METAL ION COMPLEXING PROPERIES OF AMIDE DONATING LIGANDS

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TABLE OF CONTENTS

ABSTRACT

The present state of knowledge of the coordinating properties of polyamine ligands with pendant amide groups is reviewed. The coordinating properties of ligands of this type are largely unknown at the present time. The high basicity of amide oxygen donors relative to other neutral oxygen donors such as ethers is discussed. This high basicity should make these ligands much more strongly complexing than analogs with ethereal type donor atoms such as crown ethers. The ligands EDTAM (ethylenediamine-N, N, N', N'-tetraacetamide), and NTAM (nitrilotriacetotriamide) contain neutral acetamide oxygen donors. These ligands have been synthesized by improved methods. The protonation constant for the ligand EDTAM was determined to be 4.37 in 0.1M $NaNO_3$ at 25° C. Log K values were determined with a variety of metal ions: Ca^{2+}, 3.29; Sr^{2+}, 2.30; Ba^{2+}, 2.27; La^{3+}, 5.16; Co^{2+}, 5.94; Pb^{2+}, 6.16; Cd^{2+}, 7.40; using potentiometric and polarographic methods. The protonation constant for NTAM has been determined to be 2.60 in 0.1M $NaNO_3$ at 25 ºC. Crystals were grown of the metal ion complex $[Cd(EDTAM)](NO_3)_2$. This was done to determine the mode of coordination of the amides and the number of amides coordinated to the metal ion.

ACKNOWLEDGMENTS

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Finally, I would like to thank my parents for their encouragement throughout my chemistry career, undergraduate and graduate. It is through their support and love that I have been able to accomplish all that I have today.

DEDICATION

I would like to dedicate this work to my grandmother, Janette H. Dodgin. Throughout my life she emphasized the importance of education. She taught me to set my goals high and reminded me to stick with them even when it seemed impossible to achieve my dreams. I thank her for emphasizing that I need to remember that God is in control of everything and to put Him first in everything that I do. I regret that she will not be able to read this dedication or to see me graduate. No one will ever be able to take away all of the wonderful memories and the many amazing things that I learned from her. She will forever be in my heart. Thanks, MawMaw, for all that you did and were.

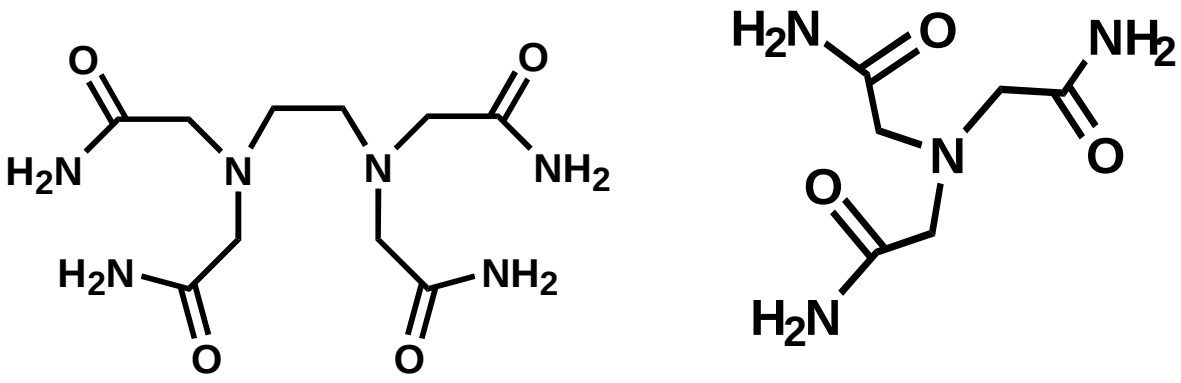
LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Ligand design is currently of great importance in coordination chemistry. The word ligand is derived from the Latin verb *ligare* meaning "to bind". In a coordination complex, the central atom (the metal) is coordinated to one or more molecules or ions (ligands). The atom in the ligand that is directly bound to the central atom or ion is called the donor atom. Metal-ligand complexes are used in a variety of applications, such as MRI (Magnetic Resonance Imaging) or radio pharmaceuticals for body imaging in medicine,[1,2] as isotopes for treatment of cancer,[3] and in the development of sensors for the distribution and movement of metal ions in living cells.[4] There has been very little attention paid to the coordinating properties of the neutral oxygen donor of amide groups with metal ions. A major part of this thesis is the study of the coordinating properties of ligands with amide coordinating groups, particularly EDTAM (ethylenediamine-N,N,N',N'-tetraacetamide) and NTAM (nitrilotriacetamide), shown in Figure 1. It is surprising that so little attention has been paid to the coordinating properties of amide oxygen donors. One must note that the amide nitrogen is completely non-basic and does not coordinate to metal ions unless it is deprotonated (Figure 2). Coordination occurs through the carbonyl oxygen of the amide group.

A literature review of structures of amide complexes of metal ions was carried out using the Cambridge Crystallographic Database.[5] This review showed a large number of publications that have appeared regarding the coordinating properties of ligands containing the similar alcoholic and ethereal neutral oxygen donors but not many with the amide oxygen donor. (See search results in the Appendix.)



a) b)

Figure 1. a) EDTAM (ethylenediamine-N, N, N', N'-tetraacetamide) and b) NTAM (nitrilotriacetotriamide)

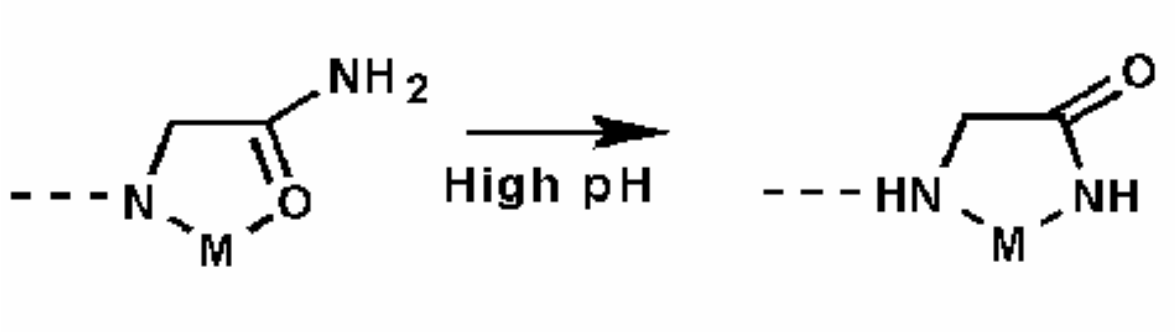


Figure 2. Amides bind to metal ions through the carbonyl oxygen. The nitrogen can become deprotonated at higher pH, and bonding switches to the nitrogen atom. (M=metal ion)

Amides donors are of considerable importance in biology,[6] where, for example, they are often the coordinating groups to metal ions. This occurs in proteins such as calmodulin,[7] annexin,[8] and parvalbumin.[9] Calcium has a major role as a second messenger within the cell. The concentration of calcium within the cell is extremely low at approximately 10^{-7} *M*. The attachment of a trigger molecule on the surface of an appropriate membrane, often the outer surface of the cell, releases Ca^{2+} into the cytoplasm of the cell. Once in the cell, the higher concentration of Ca^{2+} causes the Ca^{2+} to bind to a Ca-selective binding site on a protein. The binding of the Ca^{2+} to the protein causes the protein to change conformation, and in the new conformation, the protein binds to the enzyme that it controls, which activates the enzyme. A Ca^{2+} receptor, such as calmodulin, is used as an activator in many different situations e.g. in hormonal, neuronal, visual, and muscle stimuli. Eukaryotic cell division is regulated by Calmodulin. The Ca/calmodulin system is used as a trigger in many situations, highlighting the parsimony of nature once it has developed an efficient system. The calmodulin protein is shaped like a dumbbell with three Ca^{2+} receptors on each end (Figure 3). When Ca^{2+} binds to calmodulin, the protein folds into the dumbbell shape shown in Figure 3, and wraps around the enzyme molecule, which switches the enzyme on. Calmodulin also mediates the calcium pump that pumps the Ca^{2+} out of the cell again. The binding site in a typical calmodulin molecule involves one chelating carboxylate from glutamate, two unidentate carboxylates from asparates, two water molecules, and two amide C=O bonds (amide oxygen donors). Figure 4 shows an example of the typical binding site for calmodulin. This particular figure shows an alcoholic oxygen that is coordinated. The

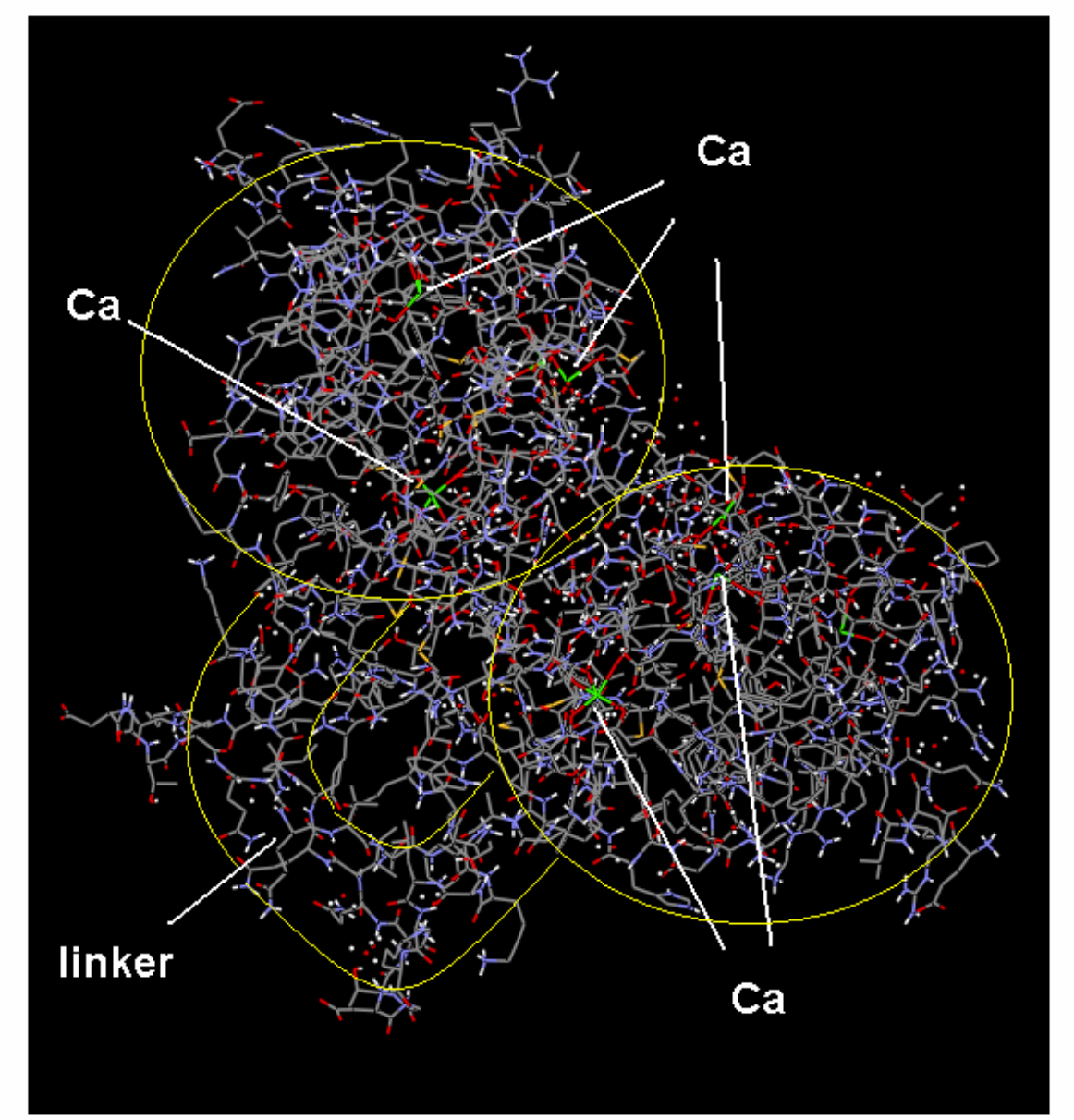


Figure 3. The structure[7] of the entire calmodulin protein showing the calcium binding sites and folding of the dumbbell shape (yellow outline) on coordination of six Ca^{2+} ions to the binding sites on the protein.

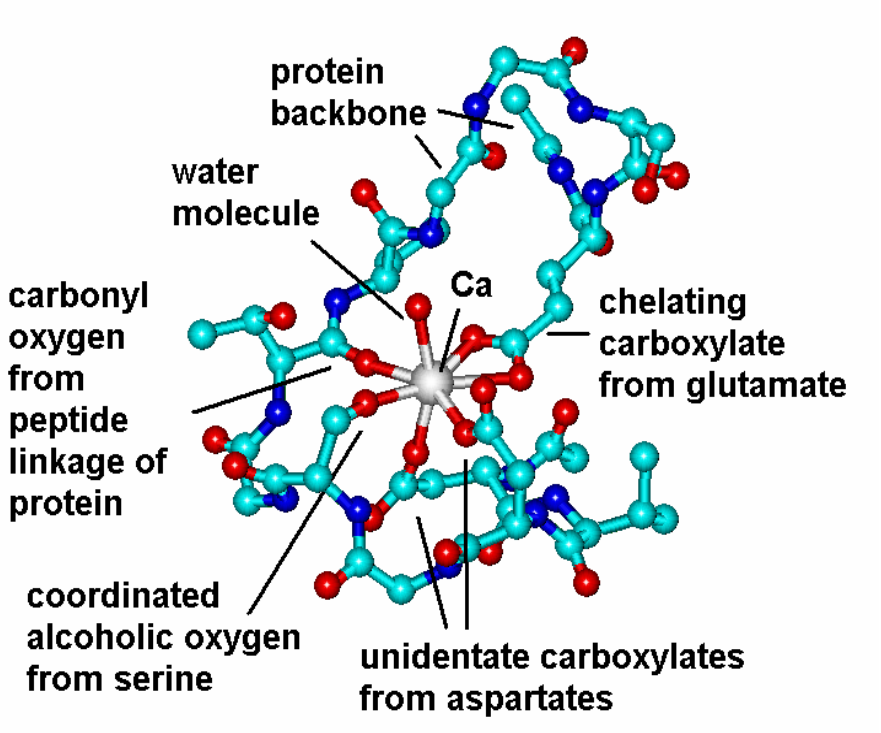


Figure 4. Structure for the binding site of calmodulin, [7] showing the coordination geometry around Ca(II). Ca(II) has a C.N. of 7 including the coordinated water molecule.

alcohol is from serine. In most calmodulin molecules, this site is normally a glutamine. The protein, annexin, (Figure 5) has three C=O bonds, as is the case with the ligand NTAM . Amide negative oxygen donors are the donor atoms lining the potassium ion channel.[7]

Hay[6] *et al*. reported the synthesis of the ligands BCE-EN (N,N'-bis(carbamoylethyl)ethylenediamine) and EDTPM (N,N,N',N'-tetrakis-(carbamoylethyl)-ethylenediamine), the structure of the BCE-EN Cu(II) complex (Figure 6), and stability constants. Since then, papers on the metal ion coordinating properties of DOTAM (1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane) have been reported[9,10] in addition to a paper[11] on an EDTAM-like ligand with N-phenyl groups attached to the amides. There have been several stability constant studies reported[6,12] on BCE-EN that show low pK_a, and moderate log K_1 values with the metal ions Cu(II), Zn(II), Ni(II), and Co(II). Some amide donor ligands have also been reported[13] where amide donors have been added to the nitrogen donors of cyclam.

The ligands reported by Hay[6] and Chung[12] have the amide groups coordinated as part of six-membered chelate rings. This is probably not detrimental for complexing small metal ions such as Cu(II). However, it has been pointed out[12] that six-membered chelate rings do not coordinate well with larger metal ions, such as Ca^{2+}, so that BCE-en is not likely to complex strongly with Ca^{2+}. Ligands such as crown ethers contain several neutral oxygen donors that are ethereal oxygens. Crown ethers complex well only with metal ions that have an ionic radius greater than about 1.0 Å. This is due in part to small metal ions being unable to coordinate to all of the oxygen donor atoms of crown ethers simultaneously, as seen in Figure 7, which shows the 18-ane N_2O_4 complex of Cu(II).

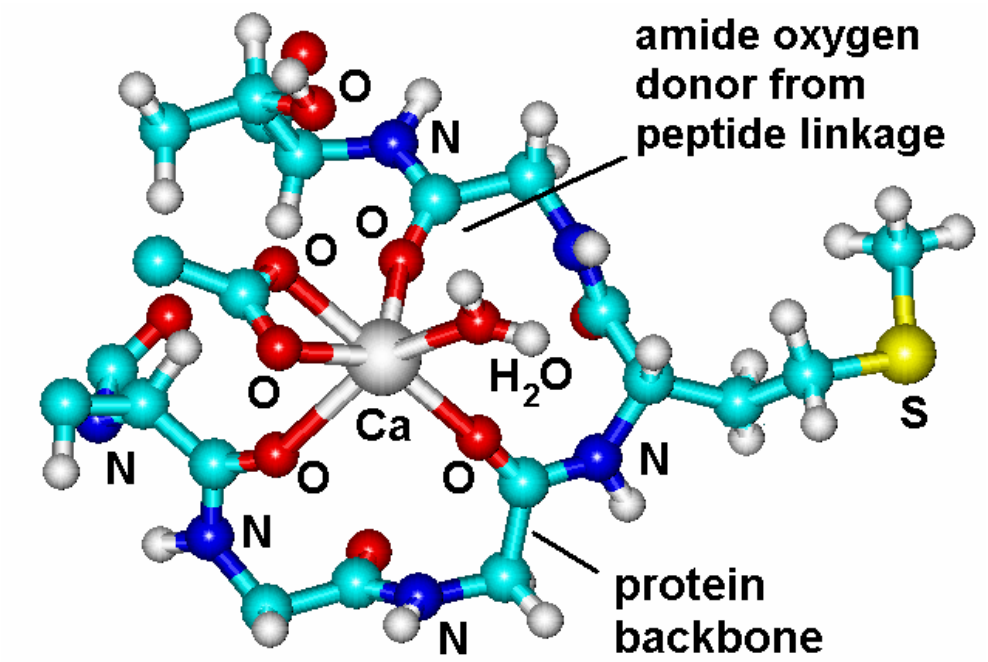


Figure 5. Structure of the binding site of annexin,[8] showing the coordination geometry around Ca(II). Ca(II) shows a C.N. of six including the coordinated water molecule.

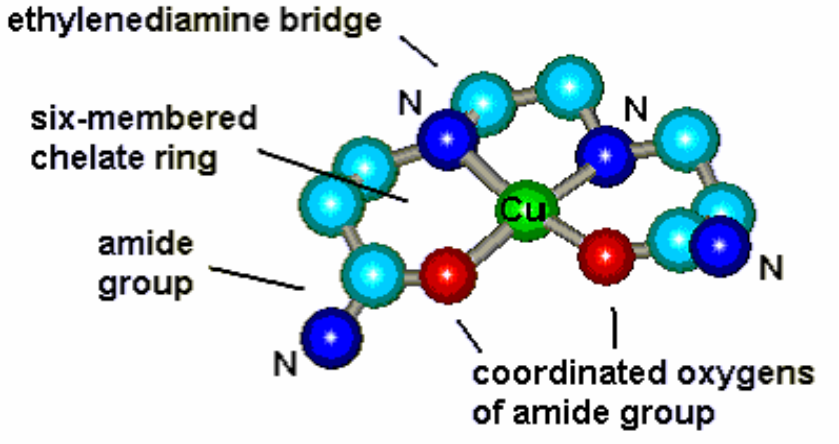


Figure 6. Cu(II) complex of BCE-EN (bis(2-carbamoylethly)ethlenediamine)[6]

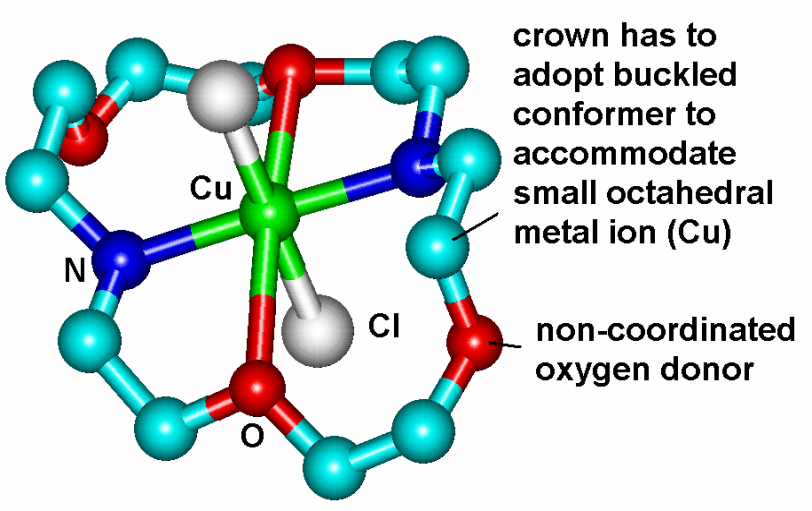


Figure 7. 18-ane N_2O_4-Cu complex

The Cu(II) is octahedral with a 2 N, 2 O, 2 Cl donor set, with two of the oxygen donors from the crown being non-coordinated to the Cu(II). In contrast, as seen in Figure 8, the large K^+ cation is able to bond to all of the donor atoms of the crown, with the crown being able to adopt the low strain D_{3d} conformer. This ability to coordinate to the crown leads to higher log *K* values for larger metal ions. Small metal ions such as Mg^{2+}, Cu^{2+}, or Ni^{2+} cannot simultaneously coordinate to all of the donor atoms of the crown and therefore form complexes of low stability. Unlike amines, the nitrogens of an amide are completely non-basic, and in all crystal structures of amides observed to date, such as in Figure 6, the coordination is via the amide oxygen. The only exception to this observation is when the pH is raised sufficiently high to cause deprotonation of the coordinated amide, thus bonding will change so that the deprotonated amide nitrogen will become coordinated to the metal ion. This happens with Cu(II) with EDTAM at approximately pH 8, but is unlikely to occur for Ca^{2+} because the bonding of Ca^{2+} to nitrogen is very weak (see Figure 2).

The ligands, EDTAM and NTAM, have acetamide donor groups, which form five-membered chelate rings and will form complexes of maximum stability with large metal ions such as Ca^{2+}. EDTAM has been reported by Przyborowski,[14] Hay,[6] and Godwin.[15] Przyborowski reported the only attempt to measure log K_1 values with EDTAM[14] complexed with Cu(II). The results were questionable, as the answers obtained seem quite out of line with expectations. It appears that in the case of the Cu(II) complex, the author mistook the deprotonation of the coordinated amide groups for the actual complexation event in the electronic UV-VIS spectroscopic studies reported. The

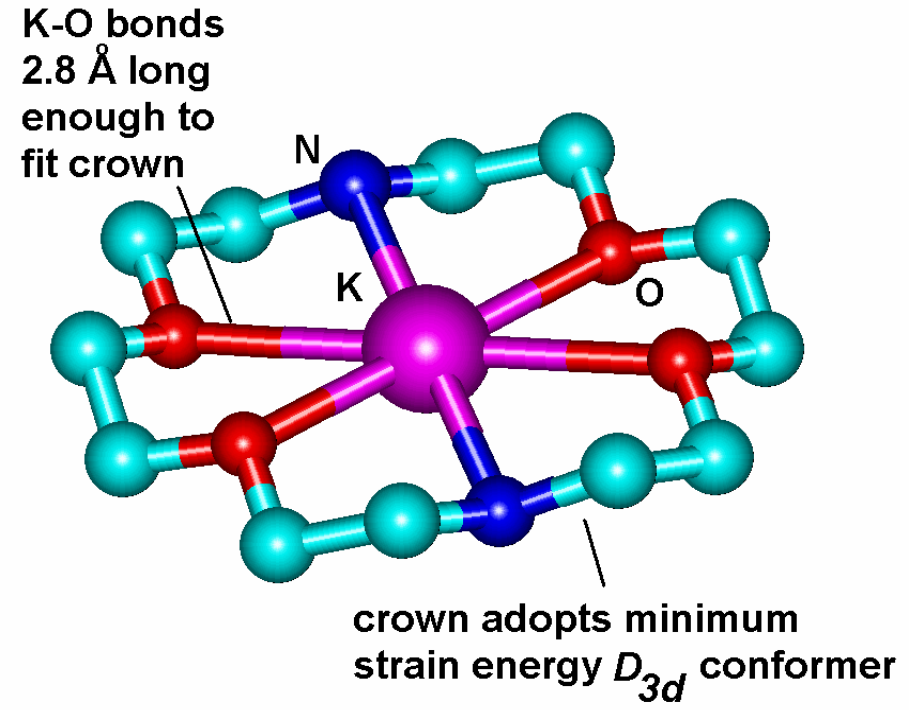


Figure 8. K-crown ether complex, showing that potassium (its atomic radius is smaller than Ca^{2+}) complexes with the crown ether

data obtained in the current research shows that the data obtained by Przyborowski was interpreted incorrectly.

An important property of the amide group is its electron-withdrawing nature. This lowers the protonation constant of the nitrogens, so that they should not be protonated at the biological pH of 7.3. This can be seen in the first protonation constants of amide- substituted ligands, which refer to protonation of the tertiary nitrogen of the ligand: (Figure 9). The ligand NTA (nitrilotriacetate) has a pK_a of 9.46. (Figure 9a) However, replacement of a single acetate group with an amide in AA-IDA lowers the pK_a by nearly 3 log units. (Figure 9b)

In EDTAM and NTAM, there are two and three amide groups per nitrogen, respectively. At the beginning of this research, it was expected that by extrapolation from the pK_a values of NTA (no amide donors) and AA-IDA (one amide donor) in Figure 9 that the pK_a value for the nitrogen of EDTAM could be as low as 4. This was determined to be true through this study of complexes of these ligands and particular metals. The main techniques used in this research for pK_a determination included potentiometry and polarography.

The main approach to studying the solution chemistry of EDTAM complexes here has been potentiometry. Potentiometry has proven to be an invaluable tool for studying solution chemistry. Potentiometry relies on measuring the potential in an electrochemical cell. This method of measurement has been useful in observing endpoints in titrations and monitoring the progress of chemical reactions. The potentiometric data generated can be used to calculate formation constants using the following equation:

$$E = E^o - \frac{RT}{nF}\ln\left[M^{n+}\right]$$

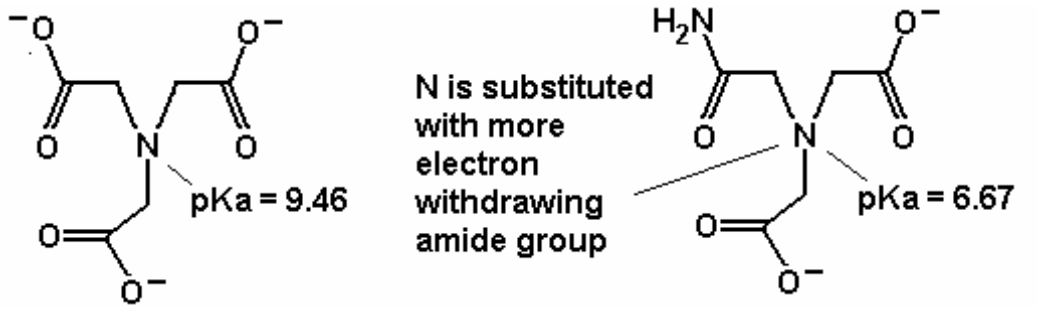


Figure 9. The effect of the electron-withdrawing amide group on nitrogen-donor pK_a. The ligand NTA (nitrilotriacetate) with its pK_a of 9.46 is shown on the left. The replacement of a single acetate group with an amide in AA-IDA lowers the pK_a by nearly 3 log units, and is shown on the right.

Formation constants are used as a measure of the stability of a complex in aqueous solution, which is important information in ligand design and understanding the functioning of metal-binding biomolecules. As the formation constant increases, the more stable the metal ligand complex becomes. A greater difference in log K_1 between the complexes of two metal ions with the same ligand indicates greater selectivity.

Glass electrode potentiometry is used to measure protonation constants by monitoring the proton concentration in the equilibrium:

$$H^+ + L = H^+L$$

(L is the ligand, e.g. EDTAM)

One combines the measured value of free $[H^+]$ in a mass balance equation to calculate the concentration of protons bound to the ligand.

$$H_T = [H^+] + [HL^+] \qquad [1]$$

$$K = [HL^+]/[H^+][L] \qquad [2]$$

At each titration point one knows L_T as well as the total acid, H_T, so that by solving the mass balance equations, one can obtain the concentrations of all the species in expression [2]. This then gives a series of values for log K_a, which usually should agree with each other within 0.05 log units. The average is taken of these values to obtain the final value of the protonation constant, log K_a, which is referred to as the pK_a. Similar experiments with other metal ions can give, by solving the appropriate mass balance equations, values of the formation constants for the complexes of the metal ions with the ligand.

Glass electrode potentiometry is the most widely used technique for determination of stability constants. However, polarography has re-entered the laboratory as a useful tool for formation constant determination. Polarography has been used in many avenues

of research such as pharmaceutical development,[16,17] drug-release study,[18,19] toxic metal ion removal,[20,21] and coordination chemistry.[21-24] Reluctance to use polarography for log *K* determination relates to the greater ease of interpretation of results from glass electrode potentiometry. However, polarography can offer advantages over potentiometry. Many formation constants obtained from polarography are inaccessible by other methods. This method has the ability to detect metals at concentrations as low as 10^{-6} M. This low-level metal ion concentration makes it advantageous in situations of low complex solubility. The ability of polarography to work at these low total metal concentrations means that the precipitation of solid hydroxides will thermodynamically occur at much higher pH values; consequently, one will not have a hydroxide precipitate at very low concentrations. This technique produces a current wave as a function of applied potential as the species being analyzed becomes reduced at the surface of a mercury drop electrode.[25]

A system where the equilibrium rate between the ligand, metal ion, and complex is slow on the polarographic time-scale is called non-labile. This slow equilibrium allows for separation of peaks, which is of great value in species identification. From this data, polarographic peaks are obtained that correspond to free metal, metal-complex, and other complex species. As the pH of the system is increased, shifts in these peaks may be obtained. Some examples of typical differential pulse polarograms[25] for a Bi^{3+}-15-aneN_4 system are shown in Figure 10. As can be seen from the polarograms, excellent separation of peaks is available with polarography, which simplifies identification of species. In (a) of Figure 10 at pH = 1.0, there is one peak which corresponds to Bi^{3+}. As

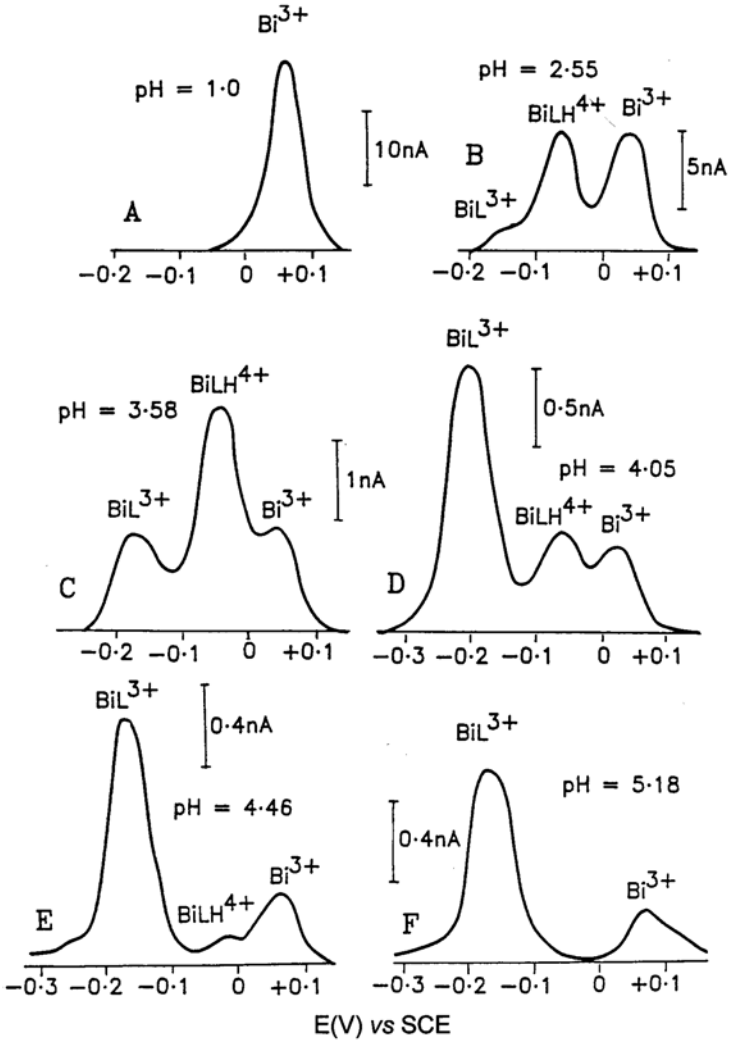


Figure 10: Differential pulse polarograms [(a) to (f)] for the Bi^{3+}-15-aneN_4 system as a function of pH[25]. This set of diagrams illustrates non-labile behavior of a metal-ligand system in polarography.

the pH increases to 2.55 in (b), a peak of $BiHL^{4+}$ and BiL^{3+} appears. At pH = 3.58 (c), the BiL^{3+} peak has increased and is the predominant peak at pH = 4.05 (d). As the pH is increased, the BiL^{3+} peak shifts with a slope of 59 mV per decade.[25]

A labile system is one in which the equilibrium is very rapid between the ligand, metal ion, and complex. These systems exhibit one peak whose potential shifts with pH as new complexes are formed. An example[25] of a labile system is shown in Figure 11, where the ligand N,N'-dipicolylethylenediamine, DPA2, with Pb^{2+} exhibits various peak potentials with increased pH. By graphing the peak potential as a function of pH, the composition of the species involved can be identified. The slopes of the *E* vs. pH relationships obey the Nernst equation.

$$E = E^o - \frac{RT}{nF} \ln\left[M^{n+}\right]$$

where *E* is the reduction potential, E^o is the standard electrode potential, *R* is the gas constant (8.316 J/mol·K), *T* is the temperature in K, *n* is the number of moles of electrons involved in the reduction process, *F* is the Faraday constant (96,485 coulombs/mole of electrons) and M is the metal ion involved in the reduction process.

The changes in slope are due to the number of protons involved in the reduction at the mercury electrode. The potential, *E*, responds to pH because the M^{n+} concentration responds to pH in equilibria such as

Low pH $\quad PbL^{2+} + 2H^+ + 2e^- \leftrightarrow Pb(Hg) + LH_2^{2+}$ (pH = 4)

High pH $\quad PbLOH^+ + H^+ + 2e^- \leftrightarrow Pb(Hg) + L + H_2O$ (pH = 11)

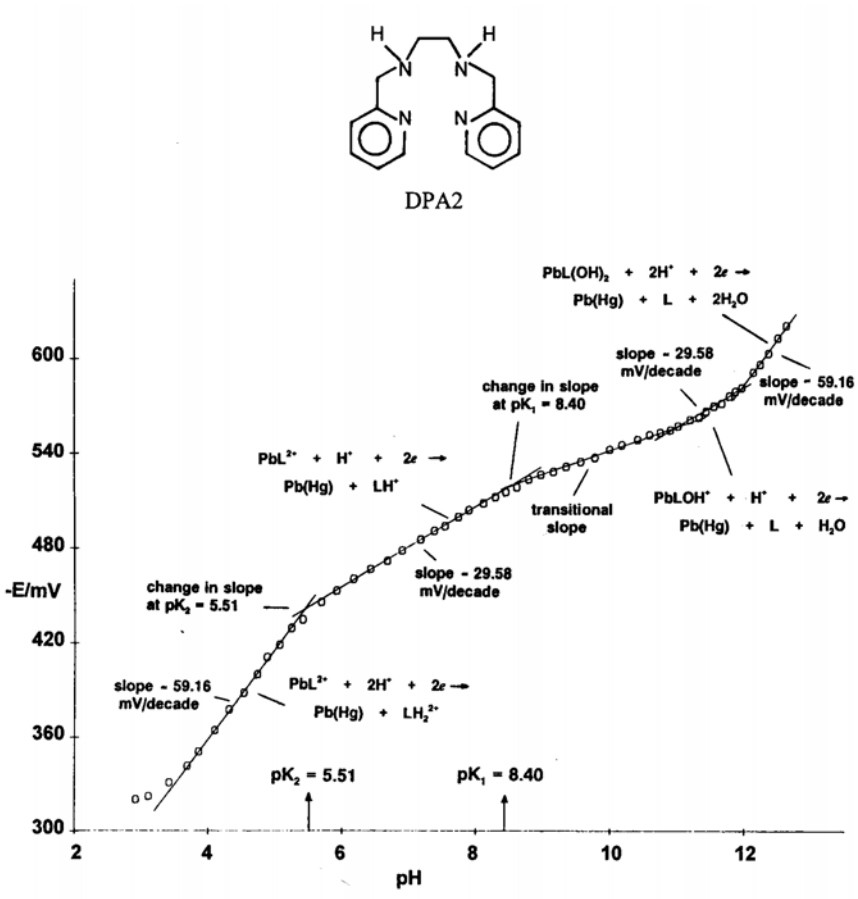


Figure 11: Variation of the polarographic peak potential (*E*) as a function of pH for DPA2-Pb complex.[25]

where L is the DPA-2 molecule (Figure 11). As the slope changes at pK_2 = 5.51, this brings about a change in slope of 29.58 which corresponds to only one proton in the reduction process. The slope changes again at pK_1 = 8.40 where there is a mix of metal-ligand and metal-ligand hydroxide complex present. Above pH = 8.40, the slope is 29.58, again indicating one proton involved in the reduction process. Once the pH rises above 12, the slope reaches a value of 59.16 mV per pH unit, which indicates the involvement of two protons in the reduction process.

The ability of polarography to study complexes at low pH and low total metal ion concentration makes this technique a valuable tool in the study of ligand-metal complexes in aqueous solution. Polarography and glass electrode potentiometry provide the main analytical tools necessary to study models of metalloenzymes in aqueous solutions.

In this study, the determination of the protonation constants and formation constants of EDTAM by glass electrode potentiometry and polarography is reported. In addition, the synthesis of $[Cd(EDTAM)NO_3]NO_3$ is reported, as well as the crystal structure of the latter determined by a collaborating group at the University of Alabama in Tuscaloosa. The results determined here for EDTAM are discussed in terms of relevance to understanding selective binding of metal ions by proteins, such as calmodulin, and implications for selectivity of small metal ions for metal ions of different binding types.

EXPERIMENTAL

All chemicals and reagents used were of analytical grade and purchased from commercial sources and used without further purification. The ligands EDTAM and NTAM were synthesized by a literature[14] known method with some modification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer. DDS in D_2O was used as an internal reference for ^{1}H measurements.

Synthesis of EDTAM

EDTAM was synthesized using a method described by Przyborowski[14] with some modifications. The synthesis was performed at half scale of that described in the paper. Ethylenediaminetetraacetic acid (19.96 g, 0.069 moles) was dissolved in 350 mL of hot absolute ethanol. Into this boiling solution, gaseous hydrogen chloride was introduced for 3 hours. Gaseous HCl was generated from the following exchange reaction between H_2SO_4 and NaCl.

$$NaCl_{(s)} + H_2SO_{4(l)} \longrightarrow HCl_{(g)} + Na_2SO_{4(l)}$$

The H_2SO_4 was dripped slowly onto solid NaCl. The apparatus consisted of a separatory funnel directly attached to a side arm flask via a rubber stopper with a single hole. Tygon® tubing connected to the side arm flask led to a glass pipette which was connected to a 3-neck flask through a thermometer adapter. This served as a bubbler to deliver the gaseous HCl to the solution of ethanol and ethylenediaminetetraacetic acid (EDTA).

This solution was refluxed for 3 hours in a hot paraffin wax bath. (Figure 12) After 3 hours of reflux, the reaction mixture was concentrated under vacuum using a Rotovap® R-3000 rotary evaporator. After this process, a vacuum pump was used to remove any residual solvent. To this product, 100 cc of water and 100 cc of ether were added while being cooled in an ice bath. While in the ice bath, this solution was neutralized with a saturated sodium bicarbonate solution. Once neutralized, the solution was extracted 5 times with 50 cc of ether each time. The combined organic layers were washed with (20mL) saturated NaCl solution and the organic layer was dried over $MgSO_4$. This solution was concentrated on the rotary evaporator until it became a viscous liquid. The ester (15.18 g) was dissolved in 200 mL of methanol containing 6 mL of liquid NH_3 (*d*. 0.6818). (Note: When adding the NH_3 to the methanol, the MeOH was cooled before addition of the liquid ammonia to avoid boiling and loss of ammonia.) This solution was mixed in a round-bottom flask and left undisturbed. After 3 days, crystals began to appear. Crystals were filtered off after 10 days. Yield was 11.2571 grams (87%). The IR was compared to an authentic sample. See results section for IR data on this compound.

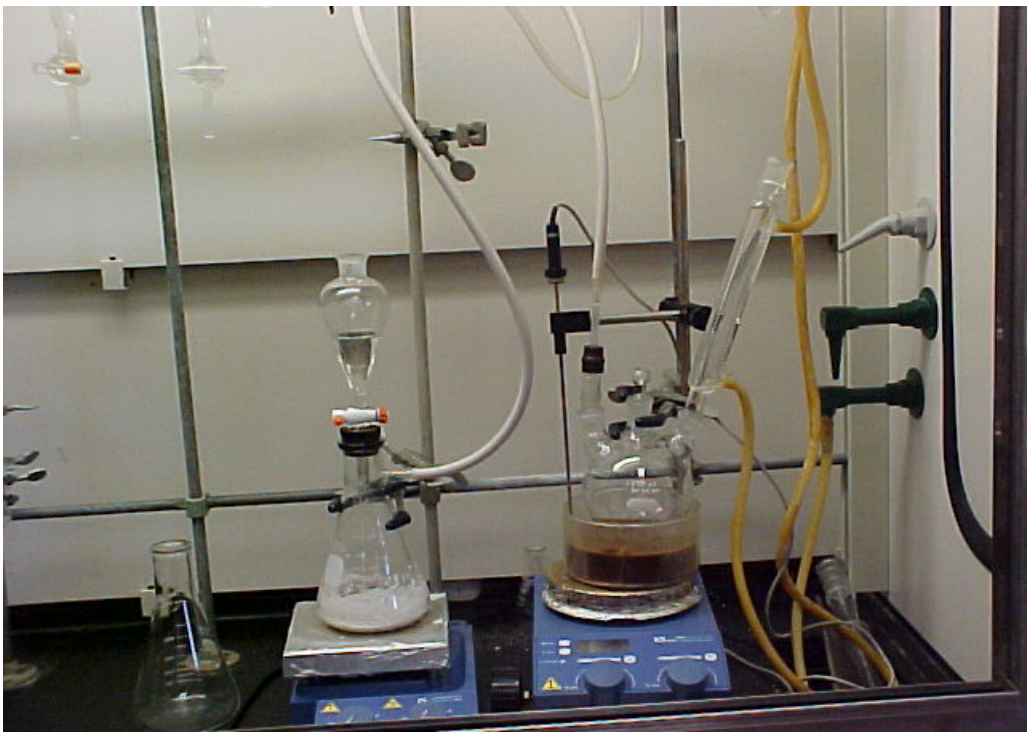


Figure 12. Ligand synthesis apparatus

Synthesis of NTAM

NTAM was synthesized using a method similar to the method for EDTAM mentioned above. 4.51g (0.024 moles) of nitrilotriacetic acid was dissolved in 200 mL of ethanol, saturated with a stream of dry hydrogen chloride and refluxed for 3 hours. The solution was then concentrated with the Rotovap® R –3000 rotary evaporator and then the vacuum pump. To this solution, 70 mL of DI water and 100 mL of ether were added, cooled, and stirred, while it was neutralized with sodium bicarbonate. Once neutralized, the solution was extracted 5 fold with 100 cc of ether each time. The combined extracts were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. This ester was dissolved in 70 cc of absolute methanol that had 2 mL of liquid NH_3 previously added. This solution was left to sit for 113 days. This length of time was not necessary because after only 21 days crystals began to appear. Yield was 2.944 g (98%).

EDTAM (Ethylenediamine-N,N,N',N'-tetraacetamide) titrations

Milli-Q® water was used to prepare all solutions. A primary standard solution (HNO_3) was used to make secondary standard solutions for all standardizations. A VWR SR60IC pH meter with an Orion PerpHecT ROSS pH electrode Model 8203BN was used for all pH and potential readings. All potential readings were measured to ±0.1 mV (±0.001 pH unit) and kept at a constant temperature of 25.00ºC ± 0.05ºC using a water circulating constant temperature bath and jacketed cell. Differential pulse voltammetry measurements were carried out using a Model 663 VA Stand (Metrohm) polarograph. This instrument was controlled by an EcoChemie PGStat 10 potentiostat as well as the

General Purpose Electrochemical System (GPES) software by EcoChemie. The multi-mode electrode was used as the working electrode in the static mercury dropping electrode (SMDE) mode. A silver/silver chloride electrode and a graphite electrode were used as the reference and auxiliary electrodes, respectively. Pulse width and integration time were set to 200 ms and 60 ms, respectively. All solutions were treated with prepurified N_2 gas to remove CO_2 and O_2 and allowed to equilibrate before each measurement.

All titrations were carried out under prepurified nitrogen at 25.00 ± 0.05°C in an airtight environment. A three-neck tapered jacketed flask was used for each titration. All solution studies were carried out at $\mu = 0.10$ ($NaNO_3$). The pH meter was calibrated daily by titrating a standard HNO_3 solution with a standardized NaOH solution. By plotting the potential vs. pH, a Nernstian slope was generated. All NaOH solutions were standardized by a previously standardized dilute solution of HNO_3.

EDTAM-Ba^{2+} log K_1 by Potentiometry

A solution of 0.01000 M EDTAM (0.2882 g in 100 mL of H_2O) and 0.1000 M $NaNO_3$ (0.8499 g in 100 mL of H_2O) was used for the titration. A solution of 0.03332 M $Ba(NO_3)_2$ (0.8708 g in 100 mL of H_2O) was used for the titration. For each potentiometric titration, the molar ratio of EDTAM:Ba was 2:1. All solutions were allowed to equilibrate for 60 minutes to ensure complete complexation of the Ba ion to the ligand, EDTAM. This solution was titrated with 1 mL additions of 0.00979 M NaOH and 0.09002 M $NaNO_3$. A one-liter stock of this solution was made. Potentials were recorded in mV when the electrode came to equilibrium.

EDTAM-Ca^{2+} log K_1 by Potentiometry

A solution of 0.01001 M EDTAM (0.2887 g in 100 mL of H_2O) and 0.1000 M $NaNO_3$ (0.8503 g in 100 mL of H_2O) was used for each titration. A solution of 0.03335 M $Ca(NO_3)_2$ (0.7875 g in 100 mL of H_2O) was used for each titration. For each potentiometric titration, the molar ratio of EDTAM:Ca was 2:1. All solutions were allowed to equilibrate for 60 minutes to ensure complete complexation of the Ca ion to the ligand, EDTAM. This solution was titrated with 1 mL additions of 0.00979 M NaOH and 0.09002 M $NaNO_3$. Potentials were recorded in mV when the electrode came to equilibrium.

EDTAM-Sr^{2+} log K_1 by Potentiometry

A solution of 0.0100 M EDTAM (0.2882 g in 100 mL of H_2O) and 0.1000 M $NaNO_3$ (0.8503 g in 100 mL of H_2O) was used for each titration. A solution of 0.03329 M $Sr(NO_3)_2$ (0.7047 g in 100 mL of H_2O) was used for each titration. For each potentiometric titration, the molar ratio of EDTAM:Sr was 2:1. All solutions were allowed to equilibrate for 60 minutes to ensure complete complexation of the Sr ion to the ligand, EDTAM. This solution was titrated with 1 mL additions of 0.00979 M NaOH and 0.09002 M $NaNO_3$. Potentials were recorded in mV once the electrode came to equilibrium.

EDTAM-Co^{2+} log K_1 by Potentiometry

A solution of 0.0100 M EDTAM (0.2882 g in 100 mL of H_2O) and 0.0900 M $NaNO_3$ (0.8499 g in 100 mL of H_2O) was used for each titration. A solution of 0.015 M $Co(NO_3)_2$ (0.4365 g in 100 mL of H_2O) was used for each titration. For each potentiometric titration, the molar ratio of EDTAM:Co was 4:1. All solutions were allowed to equilibrate for 60 minutes to ensure complete complexation of the Co ion to the ligand, EDTAM. This solution was titrated with 0.5 mL additions of 0.01923 M NaOH and 0.07998 M $NaNO_3$. Potentials were recorded in mV once the electrode came to equilibrium.

EDTAM-La^{3+} log K_1 by Potentiometry

A solution of 0.0100 M EDTAM (0.2882 g in 100 mL of H_2O) and 0.0900 M $NaNO_3$ (0.8499 g in 100 mL of H_2O) was used for each titration. A solution of 0.015 M $La(NO_3)_2$ (0.6498 g in 100 mL of H_2O) was used for each titration. For each potentiometric titration, the molar ratio of EDTAM:La was 4:1. All solutions were allowed to equilibrate for 60 minutes to ensure complete complexation of the La ion to the ligand, EDTAM. This solution was titrated with 0.5 mL additions of 0.01923 M NaOH and 0.07998 M $NaNO_3$. Potentials were recorded in mV once the electrode came to equilibrium.

Synthesis of $[Cd(EDTAM)NO_3]NO_3$

The complex $[Cd(EDTAM)NO_3]NO_3$ was synthesized from a solution containing a 1:1 (5×10^{-3} mol: 5×10^{-3} mol) ratio of EDTAM:Cd. The EDTAM (1.443 g) was dissolved in the minimum amount of hot water and added drop wise to a solution of $Cd(NO_3)_2$ (1.548 g) dissolved in hot MeOH. Solid crystalline material formed immediately. Crystals were too small for crystallography. The crystals were redissolved with a minimum amount of water and left overnight for recrystallization, remaining undisturbed at room temperature (25°C) for 24 hours. The resulting crystals were vacuum-filtered and stored under N_2 gas. X-ray crystallographic analyses of crystals were carried out by the Department of Chemistry, University of Alabama.

Voltammetry of EDTAM with Metal Ions (Pb^{2+}, Cd^{2+})

All solutions used for voltammetry were made with reagent grade chemicals and Milli-Q® water. The ionic strength of the solutions was kept constant at $\mu = 0.10$ with $NaNO_3$. In order to prevent trace metal contamination all solutions were made at time of use and all glassware was cleaned with Milli-Q® water and standardized HCl. All reaction solutions were allowed to equilibrate and degas for 10 minutes in the absence of the mercury electrode. For each titration, 50.0 mL of a solution of 5.00×10^{-5} M Cd^{2+} or Pb^{2+} in 0.09 M $NaNO_3$ and 0.01 M HNO_3 (to keep the pH at ~2, to prevent hydrolysis) was placed in a jacketed cell, and an E° reading was taken with a step potential of 0.00195 V and a modulation amplitude of –0.02505 V per second over the potential range of –0.4 V to –1.8 v. Peaks were recorded in the differential pulse (DP) mode of the instrument.

Calculation of protonation constants from potentiometric data

K_a for EDTAM and NTA was determined by titrating 50 ml of a 10^{-2} M EDTAM or NTAM in 0.1 M $NaNO_3$ solution with secondary standard 9.8752×10^{-3} M HNO_3 in the above thermostatted cell. The ionic strength of the solutions was kept constant at μ = 0.10 with $NaNO_3$. From the potentiometric data obtained it was possible to calculate values of $\bar{n}$, the total number of protons bound per ligand molecule in solution. From such an $\bar{n}$ versus pH curve, it was possible to calculate the protonation constant of the ligand. The $\bar{n}$ (L) versus log L curve for EDTAM is seen in Figure 13. The theoretical $\bar{n}$ versus pH curve can be calculated from the mass balance equation for the proton:

$$[H_T] = [H^+] + [LH^+] \quad [1]$$

where L is the ligand, H_T is the total concentration of proton in solution, and LH^+ is the monoprotonated and form of the ligand. The protonation constant, K_a is given by:

$$K_a = [HL^+]/[H^+][L] \quad [2]$$

Rearranging the protonation constant and inserting it into the mass balance equation we obtain:

$$[H_T] = [H^+] + K_a[L][H^+] \quad [3]$$

from which is obtained:

$$[H_T] - [H^+] = [L](K_a[H^+]) \quad [4]$$

Since $\bar{n}$ is defined as the ratio of total concentration of protons bound to the ligand to total ligand concentration, the following is obtained:

$$\bar{n} = ([H_T] - [H^+])/[L_T] \quad [5]$$

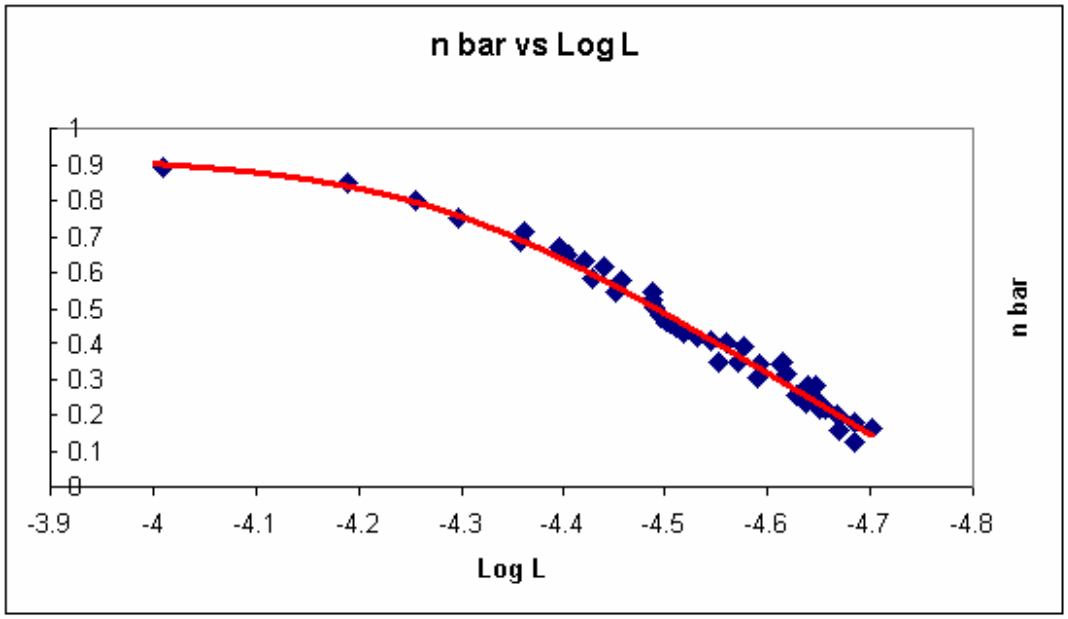


Figure 13. n bar ($\bar{n}$) versus log[L] for the La(III) EDTAM system. $\bar{n}$ is the average number of ligands bound to the metal ion for each titration point. The experimental values (◆) of $\bar{n}$ superimpose well on the theoretical curve for $\bar{n}$ versus log [L]. The value of log [L] corresponding to $\bar{n}$ = 0.5 is a rough estimate of log *K* for the system.

The expression for the mass balance equation for the ligand to can be used to calculate $[L_T]$:

$$[L_T] = [L] + [LH^+] \quad [6]$$

which on insertion of the expression for K_a (eq. 2) becomes:

$$[L_T] = [L] + K_a[L][H^+] \quad [7]$$

from which we obtain by replacing [LT] in equation [5] with equation [7]:

$$\bar{n} = (K_a[H^+])/(1 + (K_a[H^+]) \quad [8]$$

The titrations was carried out at 25.00ºC $\pm$0.05ºC, and prepurified N_2 gas was bubbled through the solution to exclude CO_2.

Calculation of log K_1 values from glass electrode potentiometry

Formation constants for metal ion complexes have the form:

$$K = [ML]/[M][L] \quad [9]$$

where [ML] is the molarity of complex ML, [M] is the molarity of free metal ion, and [L] is the molarity of free ligand (EDTAM). If, for each point, [ML], [M], and [L] can be determined, then K can be calculated. The glass electrode is used to monitor pH. The proton mass balance does not contain any metal containing species in the simplest case, so that equation [4] can be used to calculate [L], the concentration of free ligand for each titration point. The ligand mass balance in the metal-ligand titration contains the metal complex (ML) as well, as a ligand-containing species:

$$[L_T] = [L] + [LH^+] + [ML] \quad [10]$$

The K_a has now been determined as described above, and the following substitution can be made of the expression for the K_a value for the ligand (equation [2]) into equation 10:

$$[L_T] = [L] + [H^+][L]/K_a + [ML] \qquad [11]$$

For each titration point, $[H^+]$ is measured by the glass electrode, and [L] can be calculated from equation [4]. Since L_T is known for each point, then the concentration of ML can be calculated. For each titration point, one now knows [ML] and [L], and [M] can be calculated from:

$$[M] = [M_T] - [ML] \qquad [12]$$

One is therefore able to calculate a value of log K_1 for the complex ML from each titration point using equation 9. A sample of values calculated for log K_1 for Ca^{2+} with EDTAM is shown in Table 1.

One can calculate values of $\bar{n}$, the average number of ligands bound per metal ion, from the following equation, which applies to a simple system containing only ML, M, and L in equilibrium with each other. K is the formation constant for the complex ML.

$$\bar{n} = K[L]/(1 + K(L)) \qquad [13]$$

One sees that in Table 1 values of $\bar{n}$ have been calculated for each titration point. In Figure 13 a curve of experimental and theoretical values of $\bar{n}$ versus log [L] has been plotted for the La(III) complex with EDTAM. The good fit of the experimental points to the theoretical curve in Figure 13 shows the reliability of the calculated log K value.

Table 1. Glass potentiometric data obtained in the formation constant study of Ca(II) with EDTAM, as presented in an EXCEL file. The right hand column shows values of log *K* calculated from the potentiometric data obtained in the titration.

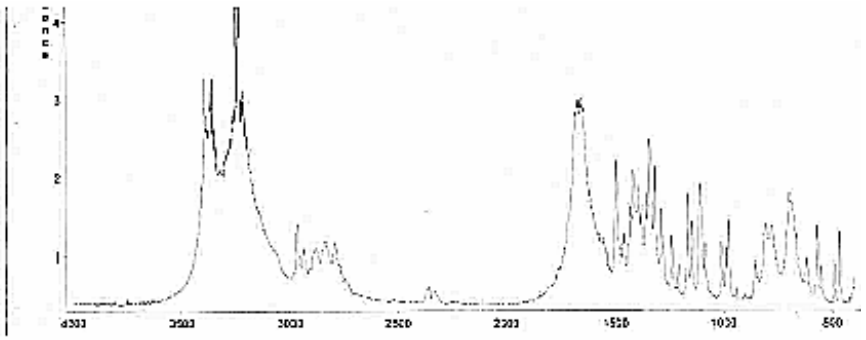
mL	mV	pH	Ht	log L	[ML]	[M]	nbar	logK
8.00	244	2.953	0.0021	-4.429	0.001448	0.016094	0.082562	3.383982
8.50	246	2.920	0.002202	-4.458	0.001406	0.015908	0.081205	3.404400
9.00	247	2.903	0.002302	-4.462	0.001322	0.01577	0.077357	3.376320
9.50	249	2.869	0.002399	-4.488	0.001298	0.015578	0.076924	3.408935
10.00	250	2.852	0.002494	-4.488	0.001227	0.015438	0.073637	3.388532
10.50	251	2.835	0.002586	-4.490	0.001162	0.015298	0.070570	3.371057
11.00	252	2.819	0.002676	-4.495	0.001101	0.015157	0.067730	3.356466
11.50	253	2.802	0.002764	-4.501	0.001046	0.015016	0.065129	3.344783
12.00	254	2.785	0.00285	-4.510	0.000996	0.014875	0.062777	3.336087
12.55	255	2.768	0.002942	-4.517	0.000941	0.014726	0.060048	3.322727
13.00	256	2.751	0.003016	-4.532	0.000912	0.01459	0.058860	3.328212
13.50	257	2.734	0.003096	-4.545	0.000878	0.014446	0.057319	3.329408
14.00	258	2.717	0.003174	-4.560	0.00085	0.0143	0.056073	3.334323
14.50	259	2.701	0.00325	-4.577	0.000826	0.014154	0.055134	3.343201

RESULTS AND DISCUSSION

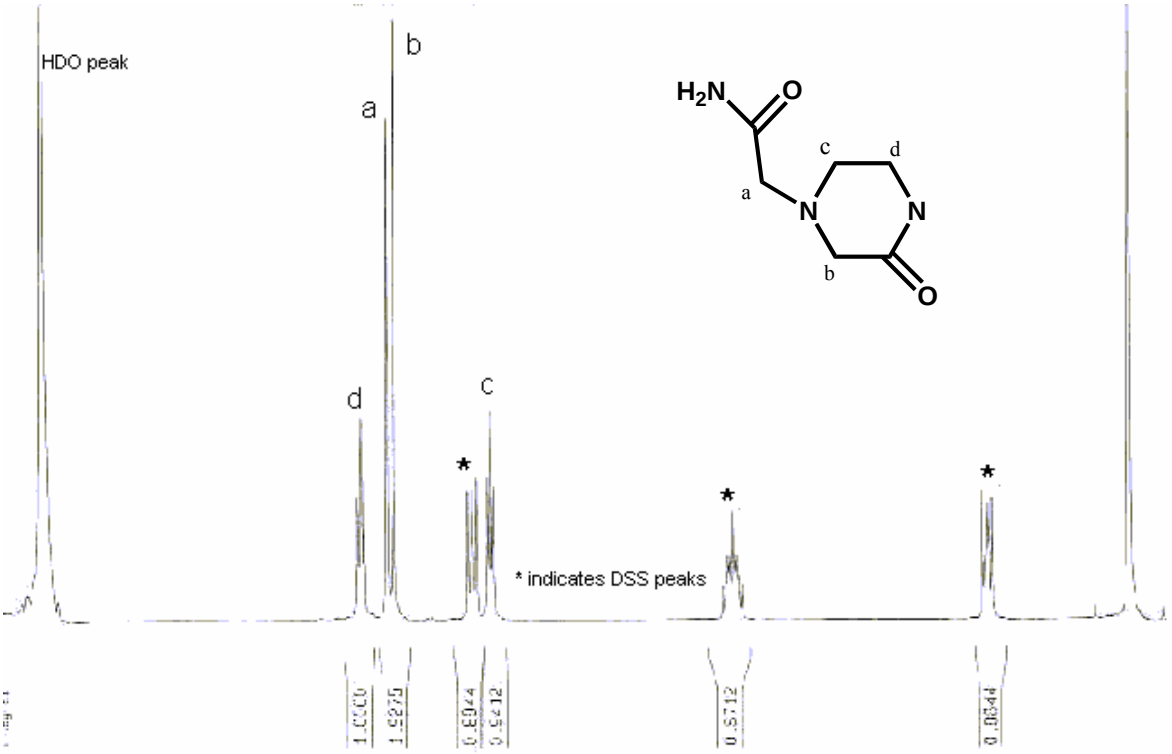
Synthesis of EDTAM and NTAM

Several attempts were made to synthesize EDTAM by a one-step method versus the conventional two-step method[14]. Several unwanted products were obtained. Using a solution containing 0.05 moles of ethylenediamine, 0.20 moles of 2-chloroacetamide, and 0.20 moles of triethylamine with ethanol as the solvent, made the product that appeared to be most similar to EDTAM. This solution was refluxed overnight. The resulting product, after NMR and IR analysis, was determined to be a lactam. (See Figure 14) After further consideration, it was decided to drip the ethylenediamine slowly into the reaction mixture. This method showed the most promise, but due to time constraints, it was decided to use the longer literature method with some modifications.

The method of Przybrowski[14] was used as a basic method to synthesize the ligands, EDTAM and NTAM. There were several modifications made to the procedural details of the EDTAM synthesis. To avoid the possibility of hydrolysis of the ester, sodium bicarbonate was used instead of the sodium hydroxide that was used in the known method. $MgSO_4$ was used as a drying agent. Crystals began to appear as soon as 3 days after completion of the process. After 10 days the crystals were filtered off to yield 9.2903 grams of product. The solution was crystallized a second time with a fresh methanol/ammonia mixture to yield an additional 1.9668 g of product. These totaled 11.2571 grams, 87% yield.



a) IR



b) NMR

Figure 14. a) IR and b) NMR of unwanted Lactam product.

Przybrowski[4] synthesized nitrilotriacetonitrile in his work as a route to nitrilotriacetic acid. The present synthesis started with nitrilotriacetic acid, which is readily available commercially. Sodium bicarbonate was also used for neutralization in this synthesis. The crystals were left for 113 days to crystallize. Crystals began to appear after 21 days, but they were not processed until needed. A total of 2.944 g (98% yield) was recovered. Infrared spectra were recorded of an authentic sample and of the product of both ligands. These were compared to determine authenticity of the product. (Figures 15 and 16) The original IR and the IR from the synthesis product matched. IR data as follows: 3413, 3383, 3296, 3245, 3199, 2862, 1674, 1649, 1578, 1468, 1413, 1343, 1257, 1116 cm^{-1}.

Potentiometric Titrations

The titration data were collected by measuring the potential (*E*) in mV using a pH meter standardized by acid-base titration performed daily. The pH was determined from the equation:

$$pH = -\log [(\text{vol. acid} \times \text{conc. acid/tot. vol.}) - (\text{vol. base} \times \text{conc. base/tot. vol.})] \quad (1)$$

Once the pH was calculated, a graph of *E* vs. pH can be shown to give a slope close to the theoretical Nernstian slope, 59.16 mV, where the number of protons and electrons in a redox process are equal. A plot of *E* vs. pH is shown in Figure 17 for an acid-base titration. Using the slope along with the previous data, the standard electrode potential, E^o, can be calculated using:

$$\frac{E^o - E}{slope} = pH \quad (2)$$

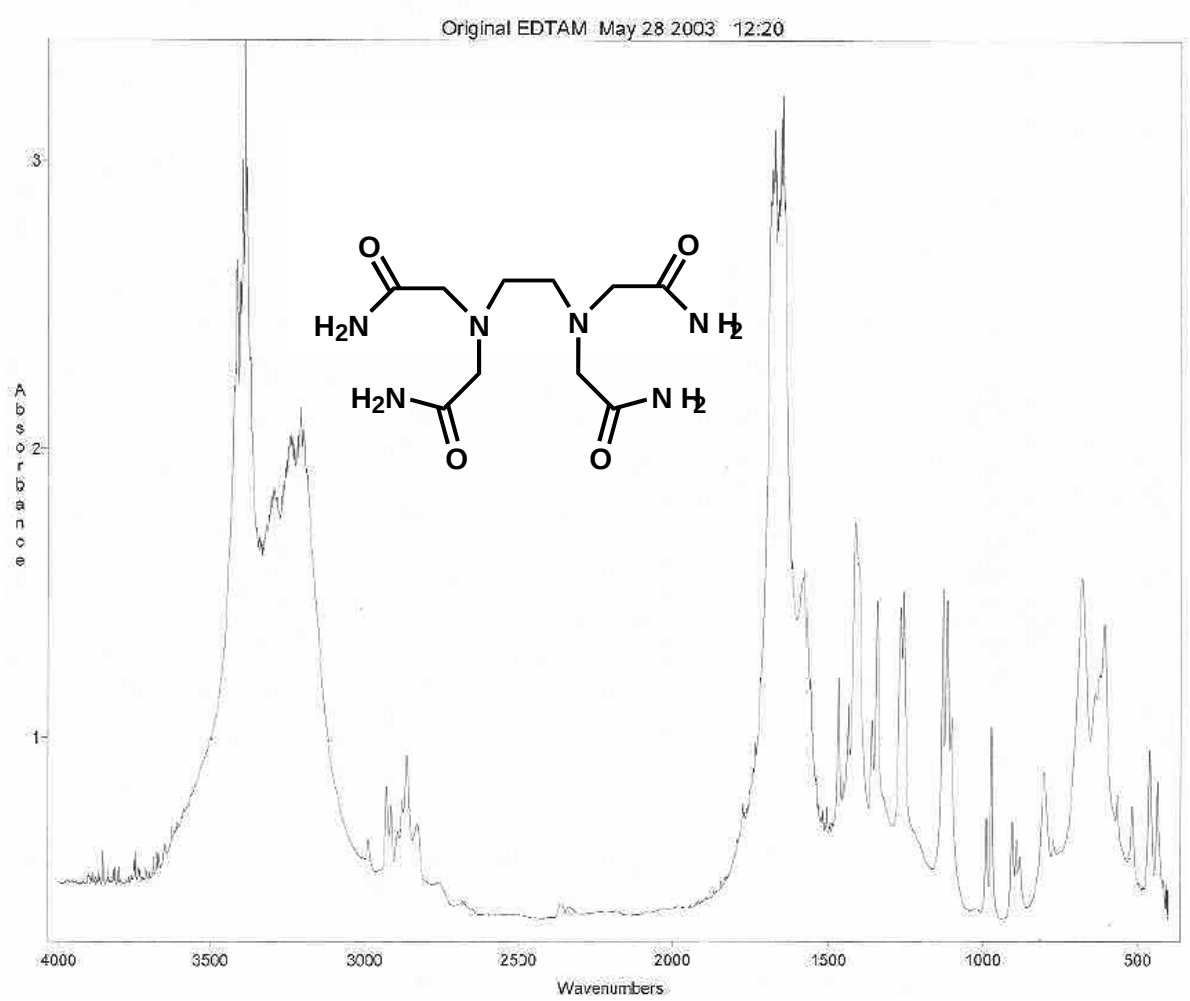


Figure 15. IR analysis of the original EDTAM sample.

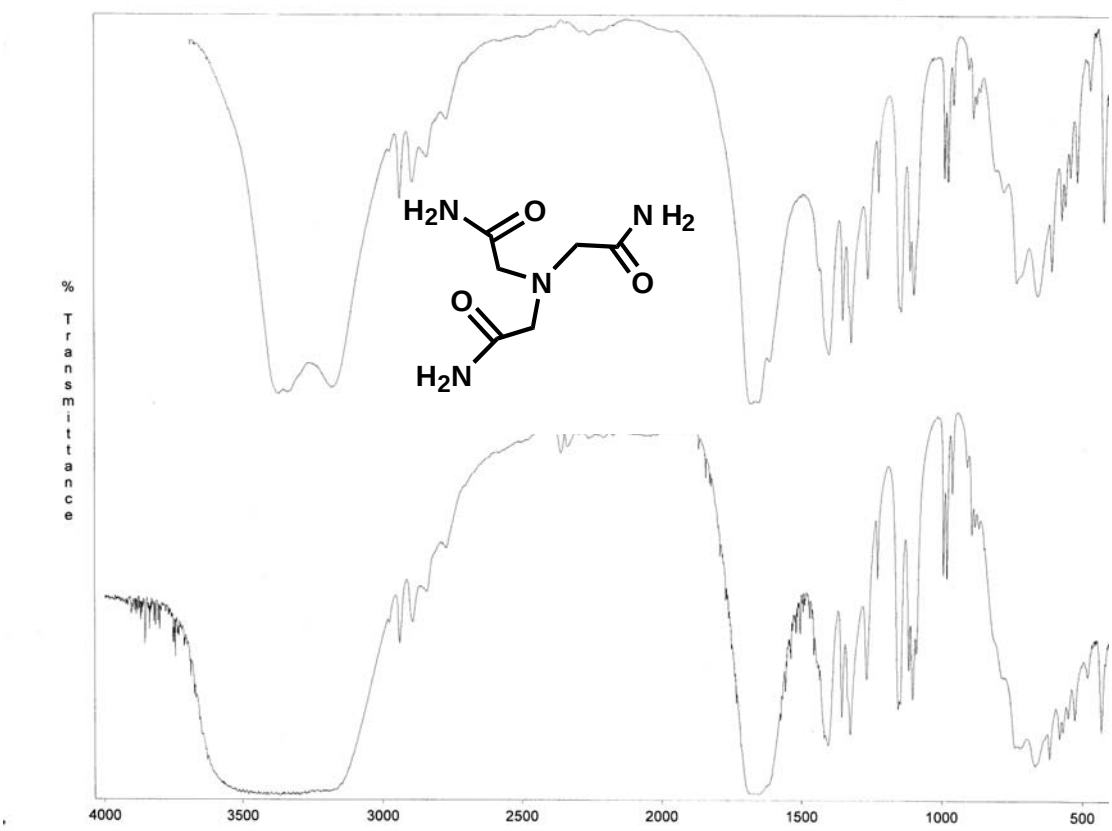


Figure 16. IR of NTAM: original and product

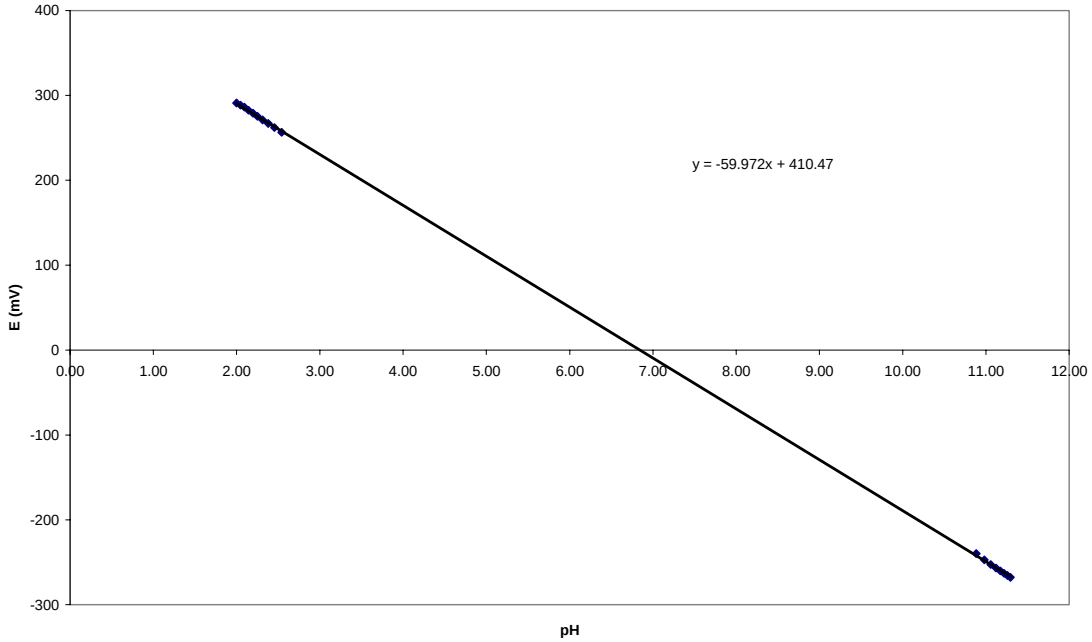


Figure 17. Plot of potential (<u>*E*</u>) in mV vs. pH for determination of E^{o} for the cell. The least squares fit of the Nernstian slope gave 59.97 mV/decade as compared to the theoretical value of 59.16 mV/decade, and an intercept = E° = 410.47 mV.

$$E^o = pH(slope) + E \quad (3)$$

The value for E^o determined for the particular cell plus glass electrode plus reference electrode used in this study ranged from 412 to 423 mV, accompanied by a small deviation of the Nernstian slope from the accepted 59.16 mV. Determined values of the Nernstian slope obtained here ranged from 57.7 to 59.9 mV/decade. It is normal for the E^o and Nernstian slope to change for a given glass electrode, which is thought to be caused by changes in the surface structure of the glass with time. This deviation in the Nernstian slope, as well as changes in E^o for the cell, are the reasons that daily determinations of these cell constants were necessary. Nernstian slopes in the range 56-61 mV are considered acceptable.

Using this primary information, a series of mass balance equations was solved in order to obtain information on the various species containing the ligand as found in solution. The three solution species containing ligand are the metal-ligand complex (ML), free ligand (L) and protonated ligand (HL). A simplifying feature of EDTAM is that above pH 2, where glass electrode potentiometry is reliable, only one protonation constant was observed. The following series of equations were used to derive the formulas used to calculate $\bar{n}$ (ratio of bound ligand to total metal concentration), as well as all species of ligand. The mass balance for the proton is give by:

$$H_T = [H^+] + [HL] \quad (4)$$

Where H_T is the total acid added to the reaction, HL is the protonated ligand, in this case, EDTAM. Inserting the rearranged protonation constant into 4 to replace [HL] gives

$$H_T - [H^+] = K_a[H^+][L] \quad (5)$$

Which can be rearranged to give [L], the only unkown in the expression as:

$$[\mathrm{L}] = \frac{(H_T - H^+)}{(K_1[H^+])} \qquad (6)$$

where K_a is the protonation constant, and $[H^+]$ is the free proton concentration measured by glass electrode potentiometry.

Once [L] is found, the concentration of metal-ligand complex (ML) can be calculated with mass balance equations for the ligand. The free ligand (L) may be calculated using the following equations

$$L_T = [L\ ] + [LH] + [ML] \qquad (7)$$

$$L_T - [ML] = L\ (1 + K_1[H^+]) \qquad (8)$$

Once both [L] and $[L_{FT}]$ are known, the only species left is complexed metal-ligand species, ML. This may be solved with equation (9)

$$[ML] = L_T - L\ (1 + K_1[H]) \qquad (9)$$

The ratio of metal-ligand complex to the total concentration of metal ion is given as $\bar{n}$. This expression relates the extent of formation of a metal/ligand complex to metal ion concentration in solution. Using the following equation, an experimental and observed value of $\bar{n}$ may be calculated

$$\bar{n} = \frac{[ML]}{\text{total conc. of metal ion}} \text{ (experimental)} \qquad (10)$$

EDTAM Results

Figure 18 shows the plot of $\bar{n}$ vs. pH for EDTAM, where $\bar{n}$ (L) is the average number of protons bound per EDTAM ligand. The potentiometric titration data was used to generate the plot. The pK_a of EDTAM can be estimated as the pH at $\bar{n}$ = 0.5. A more

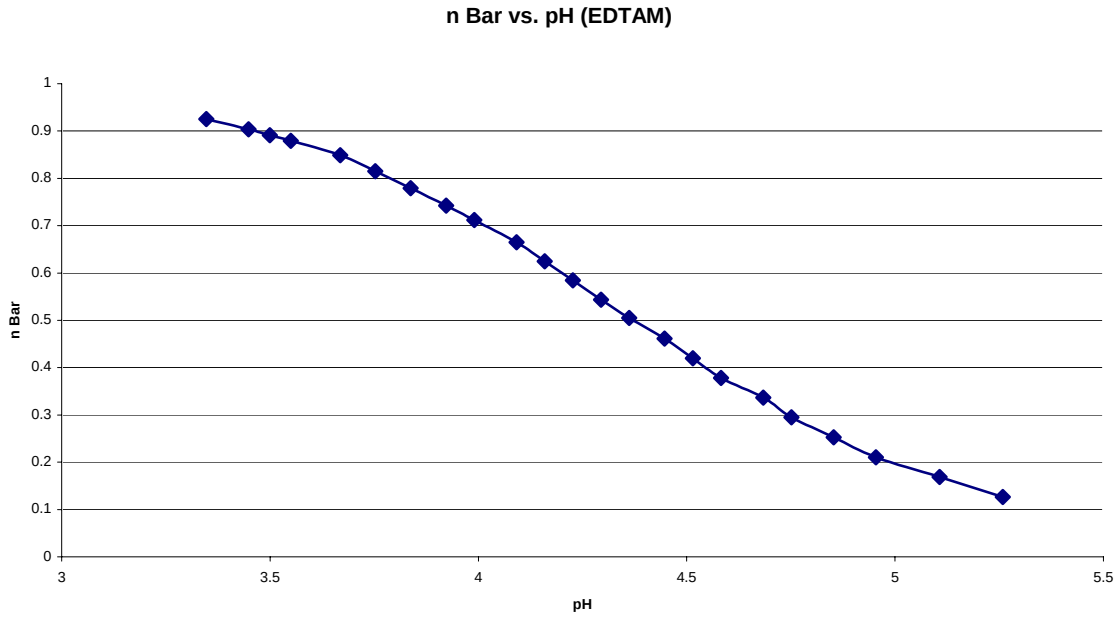


Figure 18. Plot of $\overline{n}$ (L) vs. pH for EDTAM.

accurate value of the pK_a is obtained from equation (14), calculated from each point in the curve:

$$pK_a = \log(\bar{n}\,(L)/(1-(\bar{n}\,(L))) + (pH) \quad (11)$$

The pK_a is determined to be 4.37 ± 0.02. Once the pK_a of EDTAM was determined, the formation constants, log K_1, were determined for EDTAM complexes with the metal ions Ca^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Mg^{2+}, and Co^{2+}. The Ca^{2+} and Mg^{2+} metal ions were selected due to their chemical significance in biological and biomedical systems.

Cadmium and lead are of interest in that they are toxic metal ions. Cd^{2+} and Pb^{2+} formation contants were determined polarographically. Additions of 2.0 x 10^{-4} M EDTAM were made and voltammograms recorded after each addition with the same step potential, modulation amplitude, and potential range. pH was also recorded after each addition to correct for hydrolysis if necessary. For the Cd(II) titration, twenty five 1 mL additions were made and then one 5 mL addition to make a total volume of 30 mL of ligand added to the solution. For the Pb(II), 1 mL additions were made until the volume of ligand added to the solution was 25 mL. The program EXCEL[27] was used for data storage, handling, and the execution of these calculations. The peak position and initial concentration were used with the Lingane equation[26] to determine the free metal ion concentration [M_{Free}] of the metal. (Figures 19 and 20):

$$\Delta E_p = RT/nF \ln([M_T]/[M_{Free}]) \quad (12)$$

Where ΔE_p is the shift in peak potential from the peak position where [ML] is zero, [M_T] is the concentration of total metal ion. From the value of [M_{Free}] calculated using eq. [2] for each data point, the concentration of the complex [ML] was calculated as:

$$[ML] = [M_T] - [M_{Free}] \quad (13)$$

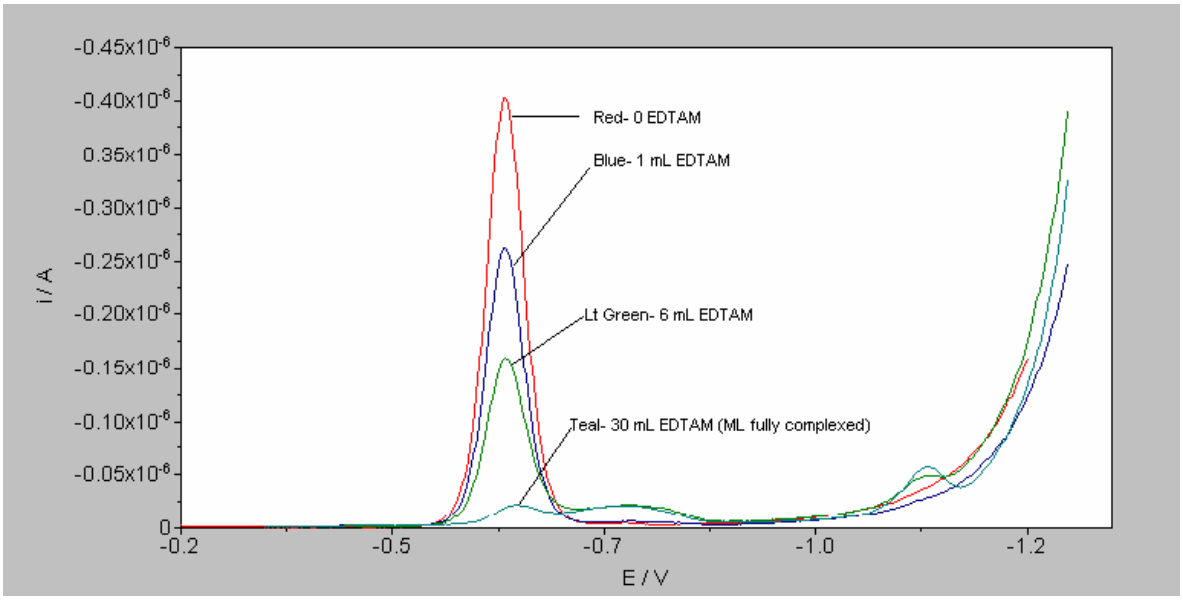


Figure 19. Polarograms for Cd(II) EDTAM system

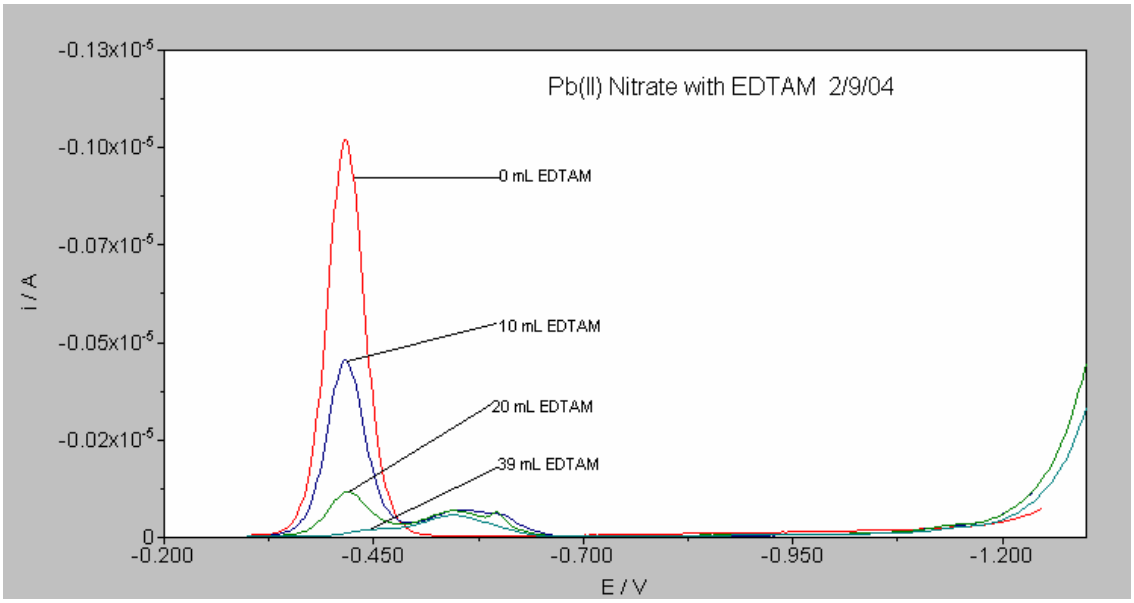


Figure 20. Polarograms for Pb(II) EDTAM system

The concentration of free ligand, [L], was calculated from the total ligand, [L_T], as:

$$[L] = [L_T] - [ML] \quad (14)$$

From the obtained values of [ML], [M_{Free}], and [L] in equations 12, 13, and 14, one can then calculate values of log K_1 for the EDTAM complexes from:

$$\log K_1 = [ML]/[M_{Free}][L] \quad (14)$$

The results for the calculation of log K_1 for Cd(II) from the polarographic peak positions are shown in Table 2. . The stability constants determined here are reported in Table 3.

Crystallographic Data of $[Cd(EDTAM)NO_3]NO_3$

Crystals were sent to Galbraith Laboratories, Inc. for C, H, and N analysis. The EDTAM-Cd complex results are as follows: Experimental: 22.58%, Carbon, 3.72% Hydrogen, and 21.09% Nitrogen, Calculated: 22.8%, Carbon, 3.83% Hydrogen, and 21.27% Nitrogen. Crystals grown of an EDTAM and $Cd(NO_3)_3$ complex were sent to the University of Alabama for crystal structure analysis. The crystal structure shows that the Cd^{2+} is complexed to the ligand through all four of the neutral oxygen donors of EDTAM. It also shows that two of the nitrate groups are bound to cadmium. This is expected because the most common coordination number for Cd^{2+} is six. Data obtained from the crystallographic determination is in Tables 4-7. The crystal structure can be seen in Figure 21.

Table 2. EXCEL sheet showing the shift in potential (E, mV) of the voltammograms for the Cd(II)/EDTAM system (0.1M $NaNO_3$, 25 °C) as a function of pH. The titration involved 50 mL of 5 x 10^{-5} M Cd^{2+} titrated with 5 x 10^{-5} M EDTAM. The mean value of log K_1 calculated is for the last 19 points of the titration. Points where $\bar{n}$ was less than 0.1 were considered less reliable and not included in the calculation.

Total volume	pH	L(total)	L(free)	E (mV)	[Cd2+]	[Cd(total)	[CdL]	K	nbar	log K
50	2.07	0	4.7994E-10	-0.582	5.00953E-05	0.00005	-9.53206E-08	-3964632	-0.00191	#NUM!
50.86	2.109	8.45458E-07	9.83873E-09	-0.582	5.00953E-05	4.92E-05	-9.40779E-07	-1908760	-0.01914	#NUM!
51.86	2.126	1.79329E-06	-3.99462E-10	-0.583	4.63437E-05	4.82E-05	1.86303E-06	-1E+08	0.038647	#NUM!
52.86	2.139	2.70526E-06	1.03533E-08	-0.583	4.63437E-05	4.73E-05	9.51059E-07	1982156	0.020109	6.297138
53.86	2.15	3.58336E-06	2.40514E-10	-0.584	4.2873E-05	4.64E-05	3.54363E-06	3.44E+08	0.076344	8.536125
54.86	2.16	4.42946E-06	1.07282E-08	-0.584	4.2873E-05	4.56E-05	2.69754E-06	5864850	0.059195	6.768257
55.86	2.165	5.24526E-06	2.10763E-08	-0.584	4.2873E-05	4.48E-05	1.88174E-06	2082488	0.042046	6.318582
56.86	2.17	6.03236E-06	1.09467E-08	-0.585	3.96622E-05	4.4E-05	4.3054E-06	9916363	0.097922	6.996352
57.86	2.184	6.79226E-06	1.80976E-09	-0.586	3.66919E-05	4.32E-05	6.5158E-06	98124275	0.150802	7.991776
58.86	2.19	7.52633E-06	1.15797E-08	-0.586	3.66919E-05	4.25E-05	5.78173E-06	13607856	0.136125	7.13379
59.86	2.209	8.23588E-06	2.88359E-09	-0.587	3.39441E-05	4.18E-05	7.82004E-06	79893559	0.187243	7.902512
60.86	2.214	8.92212E-06	1.25442E-08	-0.587	3.39441E-05	4.11E-05	7.1338E-06	16753825	0.173665	7.224114
61.86	2.219	9.58616E-06	4.07533E-09	-0.588	3.1402E-05	4.04E-05	9.01183E-06	70419518	0.222989	7.847693
62.86	2.226	1.02291E-05	1.34138E-08	-0.588	3.1402E-05	3.98E-05	8.36891E-06	19868245	0.210428	7.29816
63.86	2.233	1.08519E-05	5.52577E-09	-0.589	2.90503E-05	3.91E-05	1.00978E-05	62904869	0.257939	7.798684
64.86	2.24	1.14554E-05	1.46056E-08	-0.589	2.90503E-05	3.85E-05	9.49425E-06	22376331	0.246319	7.349789
65.86	2.247	1.20407E-05	7.23621E-09	-0.59	2.68747E-05	3.8E-05	1.10846E-05	56998536	0.292012	7.755864
66.86	2.255	1.26084E-05	1.61242E-08	-0.59	2.68747E-05	3.74E-05	1.05168E-05	24269531	0.281262	7.385061
67.86	2.259	1.31594E-05	9.18842E-09	-0.591	2.48621E-05	3.68E-05	1.19785E-05	52435198	0.325144	7.719623
68.86	2.264	1.36945E-05	1.77163E-08	-0.591	2.48621E-05	3.63E-05	1.14435E-05	25980448	0.315199	7.414647
69.86	2.272	1.42141E-05	1.14515E-08	-0.592	2.30002E-05	3.58E-05	1.27857E-05	48543290	0.357283	7.686129
70.86	2.279	1.47192E-05	1.98665E-08	-0.592	2.30002E-05	3.53E-05	1.22807E-05	26876336	0.348083	7.42937
71.86	2.284	1.52101E-05	1.39934E-08	-0.593	2.12777E-05	3.48E-05	1.35122E-05	45381484	0.388394	7.656879
72.86	2.286	1.56876E-05	3.62258E-08	-0.592	2.30002E-05	3.43E-05	1.13122E-05	13576845	0.329683	7.132799
73.86	2.291	1.61522E-05	4.44269E-08	-0.592	2.30002E-05	3.38E-05	1.08477E-05	10615949	0.320483	7.025959
74.86	2.296	1.66043E-05	5.26028E-08	-0.592	2.30002E-05	3.34E-05	1.03955E-05	8592231	0.311283	6.934106
79.86	2.318	1.86952E-05	6.30527E-08	-0.594	1.96842E-05	3.13E-05	1.16206E-05	9362825	0.371208	6.971407
										7.403377

Table 3: Log K_1 (formation constants) determined for EDTAM

Ca^{2+}	3.29	Sr^{2+}	2.30
Ba^{2+}	2.27	Mg^{2+}	~0.6
La^{3+}	5.16	Pb^{2+}	6.16
Co^{2+}	5.94	Cd^{2+}	7.40

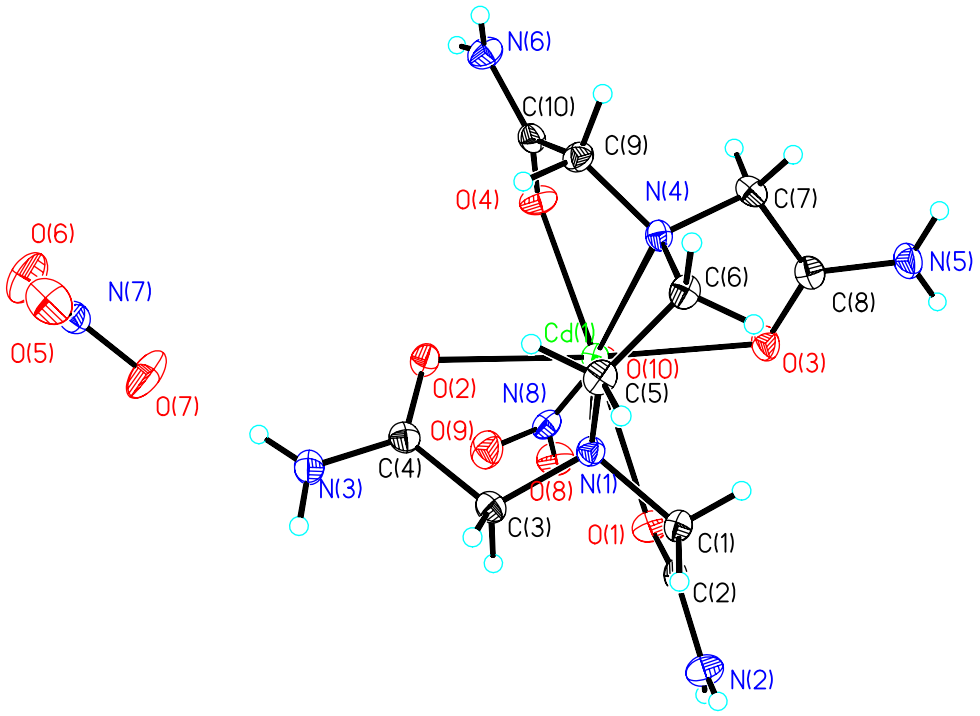


Figure 21. Crystal Structure of $[Cd(EDTAM)NO_3]NO_3$.

Table 4. Crystal data and structure refinement for $[Cd(EDTAM)NO_3]NO_3$.

Identification code	s1	
Empirical formula	C10 H20 Cd N8 O10	
Formula weight	524.74	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 10.7666(17) Å	a= 90°.
	b = 12.952(2) Å	b= 103.572(3)°.
	c = 13.273(2) Å	g = 90°.
Volume	1799.2(5) $Å^3$	
Z	4	
Density (calculated)	1.937 Mg/m^3	
Absorption coefficient	1.287 mm^{-1}	
F(000)	1056	
Crystal size	0.65 x 0.32 x 0.24 mm^3	
Theta range for data collection	1.95 to 23.27°.	
Index ranges	-11<=h<=11, -13<=k<=14, -14<=l<=12	
Reflections collected	8037	
Independent reflections	2584 [R(int) = 0.0162]	
Completeness to theta = 23.27°	100.0 %	
Absorption correction	SADABS	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2584 / 0 / 342	
Goodness-of-fit on F^2	1.083	
Final R indices [I>2sigma(I)]	R1 = 0.0167, wR2 = 0.0426	
R indices (all data)	R1 = 0.0177, wR2 = 0.0432	
Largest diff. peak and hole	0.332 and -0.397 $e.Å^{-3}$	

Table 5. Atomic coordinates ($x\ 10^4$) and equivalent isotropic displacement parameters ($Å^2 x\ 10^3$) for $[Cd(EDTAM)NO_3]NO_3$. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cd(1)	2162(1)	1706(1)	4083(1)	13(1)
O(1)	4216(1)	1866(1)	3557(1)	21(1)
O(2)	2560(1)	844(1)	5771(1)	18(1)
O(3)	1763(1)	2996(1)	2814(1)	21(1)
O(4)	118(1)	1131(1)	4169(1)	20(1)
O(5)	2552(2)	-1046(1)	9066(1)	31(1)
O(6)	1899(2)	-2467(1)	8255(1)	36(1)
O(7)	3036(2)	-1405(1)	7610(1)	36(1)
O(8)	2965(1)	-580(1)	2229(1)	25(1)
O(9)	3206(1)	-162(1)	3849(1)	27(1)
O(10)	1757(1)	595(1)	2674(1)	21(1)
N(1)	3675(2)	2640(1)	5352(1)	15(1)
N(2)	6079(2)	2741(2)	3905(2)	22(1)
N(3)	4170(2)	488(2)	7158(1)	22(1)
N(4)	939(2)	3105(1)	4624(1)	14(1)
N(5)	693(2)	4437(2)	2209(1)	21(1)
N(6)	-1452(2)	1278(2)	5016(2)	20(1)
N(7)	2488(2)	-1638(1)	8314(1)	21(1)
N(8)	2663(2)	-70(1)	2918(1)	18(1)
C(1)	4471(2)	3238(2)	4798(2)	17(1)
C(2)	4921(2)	2546(2)	4031(2)	17(1)
C(3)	4463(2)	1887(2)	6047(2)	18(1)
C(4)	3650(2)	1024(2)	6323(2)	16(1)
C(5)	2983(2)	3319(2)	5935(2)	16(1)
C(6)	1856(2)	3854(2)	5226(2)	16(1)
C(7)	173(2)	3587(2)	3680(2)	18(1)
C(8)	946(2)	3662(2)	2866(2)	17(1)
C(9)	119(2)	2637(2)	5236(2)	16(1)
C(10)	-417(2)	1616(2)	4756(2)	16(1)

Table 6. Bond lengths [Å] and angles [°] for $[Cd(EDTAM)NO_3]NO_3$.

Cd(1)-O(10)	2.3193(14)	O(10)-Cd(1)-O(3)	83.92(5)	C(9)-N(4)-C(7)	111.03(16)
Cd(1)-O(3)	2.3390(14)	O(10)-Cd(1)-O(4)	80.88(5)	C(9)-N(4)-C(6)	112.65(16)
Cd(1)-O(4)	2.3511(14)	O(3)-Cd(1)-O(4)	104.20(5)	C(7)-N(4)-C(6)	110.62(16)
Cd(1)-N(1)	2.3798(16)	O(10)-Cd(1)-N(1)	148.64(5)	C(9)-N(4)-Cd(1)	107.01(12)
Cd(1)-N(4)	2.4444(16)	O(3)-Cd(1)-N(1)	97.24(5)	C(7)-N(4)-Cd(1)	107.36(12)
Cd(1)-O(2)	2.4496(14)	O(4)-Cd(1)-N(1)	128.31(5)	C(6)-N(4)-Cd(1)	107.92(12)
Cd(1)-O(1)	2.4795(15)	O(10)-Cd(1)-N(4)	133.18(5)	O(6)-N(7)-O(5)	120.91(18)
O(1)-C(2)	1.234(2)	O(3)-Cd(1)-N(4)	70.24(5)	O(6)-N(7)-O(7)	119.00(18)
O(2)-C(4)	1.251(2)	O(4)-Cd(1)-N(4)	69.23(5)	O(5)-N(7)-O(7)	120.08(18)
O(3)-C(8)	1.246(3)	N(1)-Cd(1)-N(4)	75.08(5)	O(8)-N(8)-O(9)	122.80(17)
O(4)-C(10)	1.243(2)	O(10)-Cd(1)-O(2)	114.48(5)	O(8)-N(8)-O(10)	119.30(16)
O(5)-N(7)	1.248(2)	O(3)-Cd(1)-O(2)	161.33(5)	O(9)-N(8)-O(10)	117.90(16)
O(6)-N(7)	1.240(2)	O(4)-Cd(1)-O(2)	77.05(5)	N(1)-C(1)-C(2)	109.73(16)
O(7)-N(7)	1.254(2)	N(1)-Cd(1)-O(2)	68.97(5)	O(1)-C(2)-N(2)	123.9(2)
O(8)-N(8)	1.232(2)	N(4)-Cd(1)-O(2)	93.44(5)	O(1)-C(2)-C(1)	120.59(18)
O(9)-N(8)	1.243(2)	O(10)-Cd(1)-O(1)	80.55(5)	N(2)-C(2)-C(1)	115.52(19)
O(10)-N(8)	1.285(2)	O(3)-Cd(1)-O(1)	76.26(5)	N(1)-C(3)-C(4)	111.16(16)
N(1)-C(3)	1.467(3)	O(4)-Cd(1)-O(1)	161.26(5)	O(2)-C(4)-N(3)	123.4(2)
N(1)-C(1)	1.473(3)	N(1)-Cd(1)-O(1)	69.46(5)	O(2)-C(4)-C(3)	120.72(17)
N(1)-C(5)	1.483(3)	N(4)-Cd(1)-O(1)	126.78(5)	N(3)-C(4)-C(3)	115.92(18)
N(2)-C(2)	1.322(3)	O(2)-Cd(1)-O(1)	108.65(5)	N(1)-C(5)-C(6)	111.96(16)
N(3)-C(4)	1.316(3)	C(2)-O(1)-Cd(1)	113.44(13)	N(4)-C(6)-C(5)	111.78(16)
N(4)-C(9)	1.463(3)	C(4)-O(2)-Cd(1)	112.90(12)	N(4)-C(7)-C(8)	110.07(17)
N(4)-C(7)	1.468(3)	C(8)-O(3)-Cd(1)	117.85(12)	O(3)-C(8)-N(5)	122.03(19)
N(4)-C(6)	1.479(3)	C(10)-O(4)-Cd(1)	116.56(12)	O(3)-C(8)-C(7)	120.62(18)
N(5)-C(8)	1.316(3)	N(8)-O(10)-Cd(1)	102.97(11)	N(5)-C(8)-C(7)	117.33(19)
N(6)-C(10)	1.316(3)	C(3)-N(1)-C(1)	110.22(16)	N(4)-C(9)-C(10)	110.31(16)
C(1)-C(2)	1.518(3)	C(3)-N(1)-C(5)	110.65(16)	O(4)-C(10)-N(6)	122.7(2)
C(3)-C(4)	1.517(3)	C(1)-N(1)-C(5)	111.75(16)	O(4)-C(10)-C(9)	121.02(18)
C(5)-C(6)	1.518(3)	C(3)-N(1)-Cd(1)	107.81(12)	N(6)-C(10)-C(9)	116.21(19)
C(7)-C(8)	1.514(3)	C(1)-N(1)-Cd(1)	107.22(12)		
C(9)-C(10)	1.521(3)	C(5)-N(1)-Cd(1)	109.05(11)		

Table 7. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for $[Cd(EDTAM)NO_3]NO_3$. The anisotropicdisplacement factor exponent takes the form:
$-2p^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Cd(1)	15(1)	14(1)	12(1)	-1(1)	4(1)	0(1)
O(1)	20(1)	23(1)	20(1)	-4(1)	7(1)	-3(1)
O(2)	20(1)	18(1)	16(1)	1(1)	4(1)	-3(1)
O(3)	28(1)	21(1)	18(1)	5(1)	11(1)	7(1)
O(4)	19(1)	21(1)	22(1)	-7(1)	8(1)	-3(1)
O(5)	36(1)	31(1)	25(1)	-9(1)	3(1)	7(1)
O(6)	50(1)	29(1)	33(1)	-3(1)	17(1)	-16(1)
O(7)	52(1)	26(1)	41(1)	2(1)	34(1)	-3(1)
O(8)	29(1)	23(1)	25(1)	-10(1)	11(1)	3(1)
O(9)	31(1)	32(1)	18(1)	2(1)	5(1)	-2(1)
O(10)	19(1)	19(1)	26(1)	-4(1)	8(1)	2(1)
N(1)	15(1)	15(1)	14(1)	1(1)	4(1)	0(1)
N(2)	20(1)	24(1)	22(1)	-4(1)	8(1)	-2(1)
N(3)	20(1)	22(1)	22(1)	7(1)	2(1)	-2(1)
N(4)	17(1)	14(1)	12(1)	1(1)	3(1)	1(1)
N(5)	25(1)	21(1)	18(1)	6(1)	7(1)	5(1)
N(6)	22(1)	19(1)	21(1)	-4(1)	10(1)	-2(1)
N(7)	20(1)	21(1)	20(1)	2(1)	5(1)	4(1)
N(8)	20(1)	16(1)	20(1)	-1(1)	8(1)	-4(1)
C(1)	18(1)	15(1)	20(1)	3(1)	5(1)	-1(1)
C(2)	18(1)	18(1)	15(1)	7(1)	4(1)	2(1)
C(3)	16(1)	20(1)	16(1)	2(1)	1(1)	0(1)
C(4)	18(1)	16(1)	16(1)	-1(1)	7(1)	4(1)
C(5)	19(1)	16(1)	14(1)	-3(1)	4(1)	-3(1)
C(6)	20(1)	12(1)	15(1)	-3(1)	7(1)	-1(1)
C(7)	17(1)	18(1)	17(1)	1(1)	2(1)	4(1)
C(8)	19(1)	16(1)	13(1)	-2(1)	0(1)	-1(1)
C(9)	18(1)	18(1)	15(1)	0(1)	7(1)	0(1)
C(10)	15(1)	18(1)	13(1)	2(1)	2(1)	2(1)

CONCLUSIONS

The role of Ca^{2+} as a second messenger in biology involves selective binding[5] to sites in proteins such as calmodulin, annexin, and troponin-C.[7, 8, 28] These proteins are switches triggered by Ca^{2+} when it enters the cytoplasm of the cell as a result of the opening of calcium ion channels. It is vital that Mg^{2+}, present in higher concentration in the cell, not bind strongly to these sites and interfere with triggering by Ca^{2+}. Falke *et al*.[29-31] reported interesting studies of binding of Ca^{2+} to bacterial proteins that have sites resembling those of calmodulin, which show[29] selectivity for Ca^{2+} over Mg^{2+} of about 10^4. The possible origin[29-31] of such selectivity based on a rigid cavity containing the metal receptor site has been investigated. A rigid cavity might distinguish between the large Ca^{2+} ion, with an ionic radius (r^+)[8] of 1.00 Å, compared to the small Mg^{2+} ion with $r^+ = 0.74$ Å. Ordinarily, proteins distort easily,[29, 30, 32] typically taking, for example, about 0.15 kcal.mol^{-1} to expand the radius of a cavity from 0.9 to 1.1 Å. There might be[29, 30] some special rigidity in the cavities that contain Ca^{2+} in calcium-binding proteins to account for the selectivity for Ca^{2+} over Mg^{2+}. Site-directed mutagenesis of such proteins has been carried out[31] to successively replace several amino acid residues by glycine in the vicinity of the Ca^{2+} binding site. These were residues that might promote unusual rigidity, so that[31] change to glycine residues should produce lowered rigidity. Weakened rigidity should reduce selectivity for Ca^{2+} over Mg^{2+}. Such changes in the protein have little effect[31] on selectivity or Ca^{2+} binding strength, suggesting[31] that selectivity for Ca^{2+} over Mg^{2+}, at least in this situation, is not principally governed by unusual rigidity of binding cavity. Factors other than a rigid cavity containing the binding site could be

acting in such proteins. The importance has been recognized[29-31] of charged groups in binding sites, which act to exclude cations of low charge from sites with a larger number of negatively charged groups. In a number of proteins with Ca^{2+} binding sites, two additional themes are observable. First, there is at least one chelating carboxylate group, as seen in Fig. 23, where the binding site of Ca^{2+} in annexin is shown. As has been discussed extensively[34], small chelate rings bind with less steric strain to larger metal ions, so that it seems possible that these small four-membered chelate rings promote selectivity for the large Ca^{2+} over the small Mg^{2+} cation. Second, which is the topic of interest here, there are one to three amide O-donor atoms coordinated to the Ca^{2+} (Fig. 22), from peptide linkages of the protein backbone, or from amide groups on asparagine and glutamine residues.

To investigate the metal binding properties of the amide donor, the complexes of EDTAM (Fig. 23) have been studied. EDTAM has been reported by other workers[6, 35] but not its formation constants (log K_1) with metal ions. The usual coordination of amides through the carbonyl oxygens to a metal ion, in this case for the EDTAM complex of Pb(II), except at higher pH, has been shown crystallographically.[35] Several ligands with one or two amide groups are reported in the compilation of Smith and Martell,[36] but there are several types of donor atom present in each of these ligands, so that it is not easy to distinguish the role of the amide oxygen donors. EDTAM has four pendant amide donors attached to an *en* ligand. Mg^{2+} and Ca^{2+} have a low and approximately equal log $K_1$with *en*[36], so that differences in log K_1 with EDTAM with these ions can be reasonably attributed to differences in affinity for the amide donors.

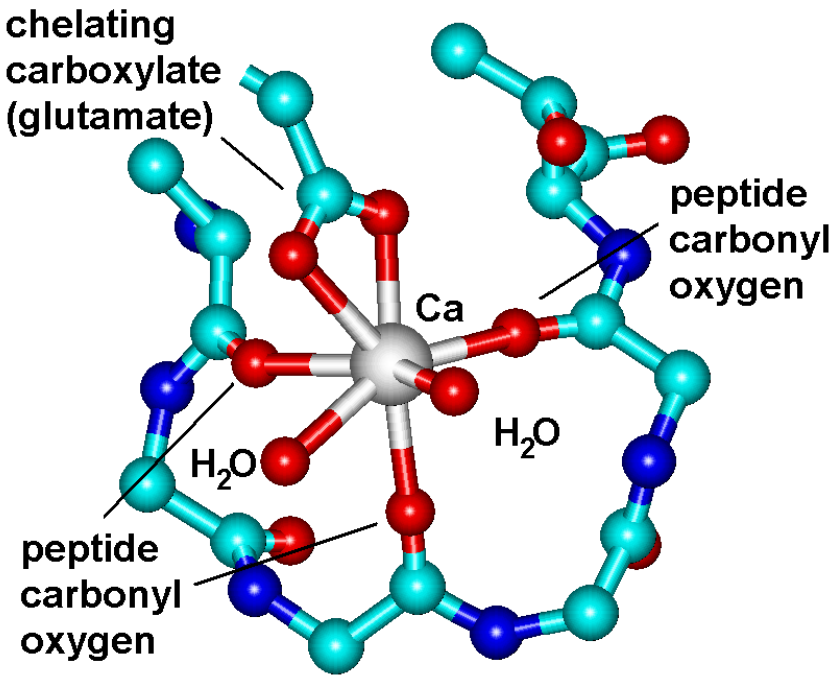


Figure 22. Binding site of Ca^{2+} in annexin, drawn with coordinates from ref 3. The Ca^{2+} is seven coordinate, held in the binding site by a chelating carboxylate from a glutamate residue, plus three amide oxygens derived from peptide linkages of the protein backbone. Two coordinated water molecules make up the rest of the coordination sphere.

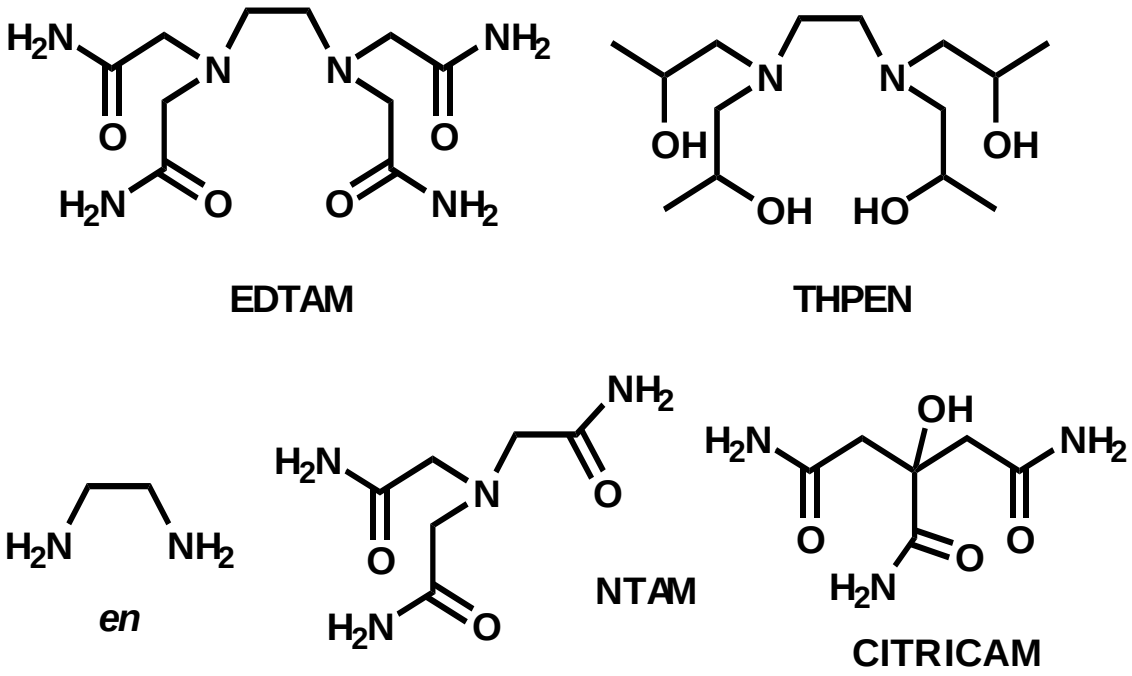


Figure 23. Ligands discussed in this work.

EDTAM was synthesized as reported.[6] Log K_1 values were determined by glass electrode potentiometry.[37] The protonation constants and log K_1 for EDTAM with Mg^{2+} and Ca^{2+}, as well as several other metal ions, are shown in Table 8, together with log K_1 values[36] for THPEN and *en* for comparison.

Table 8 shows that the amide O-donors on EDTAM produce selectivity for Ca^{2+} over Mg^{2+} of almost 10^3. This, combined with the effects of the four-membered chelate rings formed by acetates, may account for part of the selectivity for Ca^{2+} over Mg^{2+} of about 10^4 found for Ca-binding sites[29]. Log K_1 values for EDTAM are larger than for THPEN, which is analogous, but has hydroxyalkyl O-donors[38] in place of amide O-donors in EDTAM. Neutral O-donors can vary widely[39] in their strength as Lewis bases. Amide donors (Table 8) are stronger Lewis bases towards larger metal ions such as Ca^{2+} than are alcoholic or water-derived O-donors. Log K_1 values for EDTAM and THPEN give some insight into how alcoholic versus amide donors might affect Ca^{2+} binding strength and Ca^{2+}/Mg^{2+} selectivity. The Ca-binding protein calpain, for example, has EF-hand binding sites similar to those of calmodulin, except that in one a hydroxyalkyl O-donor from a serine[40] residue binds to Ca^{2+} in place of an amide O-donor from an asparagine. The log K_1 values for EDTAM and THPEN suggest that the alcoholic oxygen from a serine would lower the Ca^{2+} binding strength of the serine containing site in calpain. More weakly binding 'empty' (no Ca) EF-hand sites in troponin-C contain[40] serine in place of asparagine.

Amide O-donors are the sole K^+ complexing groups[41] in K^+ ion channels, and are likely to occur in Ca^{2+} and Na^+ ion channels[42]. A view of a K^+ ion channel is seen in

Table 8. Formation constant for EDTAM, THPEN, and *en*[a].

Lewis acid:	Ca^{2+}	Mg^{2+}	Sr^{2+}	Ba^{2+}	La^{3+}	Co^{2+}	H^{+}	references
Ionic radius (Å):	1.00	0.74	1.18	1.36	1.03	0.72	-	44
Log K_1 EDTAM:	3.29	≤0.6	2.30	2.15	5.19	5.94	4.36	this work
Log K_1 THPEN:	1.63	≤0.3	0.8	~0	2.90	6.1	8.67	38
Log K_1 *en*:	0.11	0.37	-	-	(1.4)[b]	5.5	9.92	36

[a]25°C and ionic strength 0.1 ($NaNO_3$). [b]Estimated in reference 38.

Figure 24. Studies of ligands containing amide donor groups could provide further insight into the metal-binding properties of proteins utilizing amide donors. The saturated N-donor, as found in EDTAM, reduces[36,43] the affinity of ligands for Na^+ and K^+, and EDTAM does not appear to bind to Na^+ or K^+. NTAM has a weak contribution to binding from its N-donors. The amide groups appear to be very electron-withdrawing, and NTAM has a pK_a of only 2.6, which might improve binding to Na^+ and K^+. In order to remove the N-donor altogether, the aim is to study the metal binding properties of ligands such as CITRICAM (Figure 23), and other ligands containing amide O-donors only.

The importance of Ca^{2+} over Mg^{2+} recognition in a host of calcium-binding proteins cannot be overstated. It is perhaps surprising, in view of the widespread occurrence of amide donors in metal ion binding sites in biology, that no studies have been reported of small ligands that would allow evaluation of the strength of the amide donor, and its ability to discriminate between metal ions on the basis of size. This study reports, the donor properties of the amide-donor ligand would be dominant, at least for Ca^{2+} and Mg^{2+}. It also shows that it is likely that intrinsic affinity of the amide group for Ca^{2+} over Mg^{2+} accounts for much of the selectivity for Ca^{2+} displayed by these proteins. This is a radical departure from current thinking, which is based on ideas derived from crown ether and cryptand chemistry, where discrimination is produced by a rigid cavity. In retrospect, it seems logical that nature would not use a rigid cavity, as this could lead to slow on-off times for the Ca^{2+} binding to the protein. It is perhaps surprising, in view of the widespread occurrence of amide donors in metal ion binding sites in biology, that

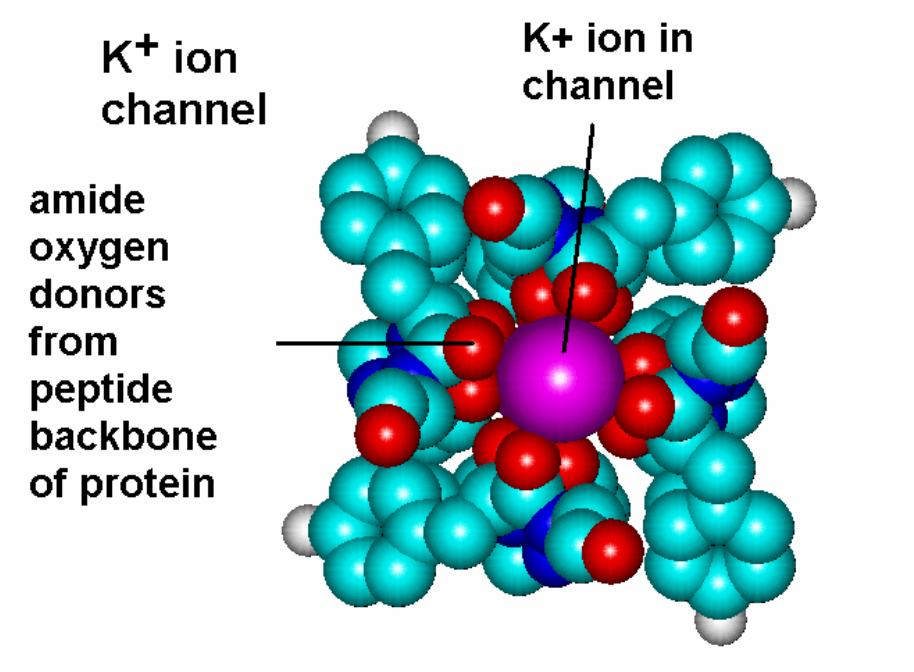


Figure 24. View of the potassium ion channel[41] showing a potassium ion held by neutral oxygen donors of the amide type derived from the peptide bonds of the protein.

no studies have been reported of small ligands that would allow evaluation of the strength of the amide donor, and its ability to discriminate between metal ions on the basis of size.

This thesis reports the first such study, where the donor properties of the amide-donor ligand would be dominant, at least for Ca^{2+} and Mg^{2+}. The study shows that it is likely that intrinsic affinity of the amide group for Ca^{2+} over Mg^{2+} accounts for much of the selectivity for Ca^{2+} displayed by these proteins. This is a radical departure from current thinking, which is based on ideas derived from crown ether and cryptand chemistry, where discrimination is produced by a rigid cavity. In retrospect, it seems logical that nature would not use a rigid cavity, as this could lead to slow on-off times for the Ca^{2+} binding to the protein.

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APPENDIX

COJHOZ

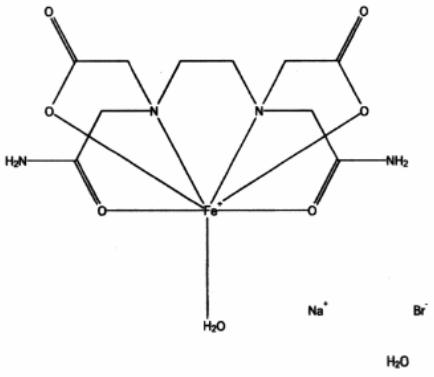
Reference: N.K.Diop, L.K.Vitellaro, P.Arnold, Macyu Shang, R.A.Marusak (2000) *J.Inorg.Biochem.*,78,209

Formula: $2(C_{10}H_{18}Fe_1N_4O_7^{1+}),Na_1^{1+},3(Br_1^{1-}),2(H_2O_1)$

Compound Name: bis(Aqua-(N,N'-bis((carbamoyl)methyl)-1,2-diaminoethane-N,N'-diacetato)-iron(iii)) sodium tribromide dihydrate

Synonym: bis(Aqua-(N,N'-ethylene-bis(N,N'-bis((carbamoyl)methyl)glycinato))-iron(iii)) sodium tribromide dihydrate

R-Factor (%): 3.26 **Temperature(K):** 293 **Density(g/cm³):** 1.872



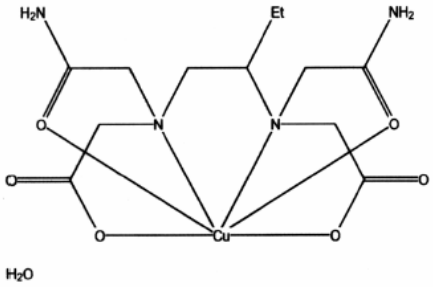
EEMGCU

Reference: C.K.Prout, D.S.Sanderson, M.C.Couldwell (1979) *Cryst.Struct.Commun.*,8,181

Formula: $C_{12}H_{20}Cu_1N_4O_6,2(H_2O_1)$

Compound Name: 1-Ethylethylene-1,2-bis(N-methylcarbamyl-glycinato)-copper(ii) dihydrate

R-Factor (%): 4.80 **Temperature(K):** 295 **Density(g/cm³):** 1.627



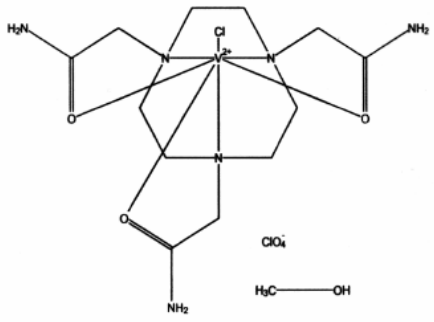
GIWRAF

Reference: T.Weyhermuller, K.Wieghardt, P.Chaudhuri (1998) *J.Chem.Soc.,Dalton Trans.*,3805

Formula: $C_{12}H_{24}Cl_1N_6O_3V_1^{2+},2(Cl_1O_4^{1-}),2(C_1H_4O_1)$

Compound Name: (1,4,7-tris(Carbamoylmethyl)-1,4,7-triazacyclononane)-chloro-vanadium(iii) diperchlorate methanol solvate

R-Factor (%): 5.28 **Temperature(K):** 100 **Density(g/cm³):** 1.676



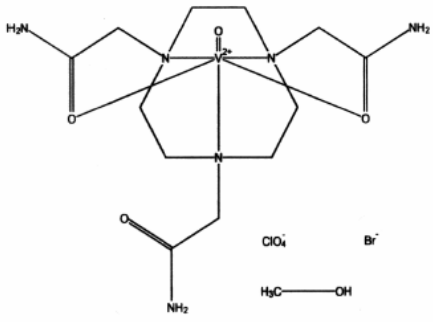
GIWREJ

Reference: T.Weyhermuller, K.Wieghardt, P.Chaudhuri (1998) *J.Chem.Soc.,Dalton Trans.*,3805

Formula: $C_{12}H_{24}N_6O_4V_1^{2+},Cl_1O_4^{1-},Br_1^{1-},C_1H_4O_1$

Compound Name: (1,4,7-tris(Carbamoylmethyl)-1,4,7-triazacyclononane)-oxo-vanadium(iii) bromide perchlorate methanol solvate

R-Factor (%): 3.62 **Temperature(K):** 100 **Density(g/cm³):** 1.777



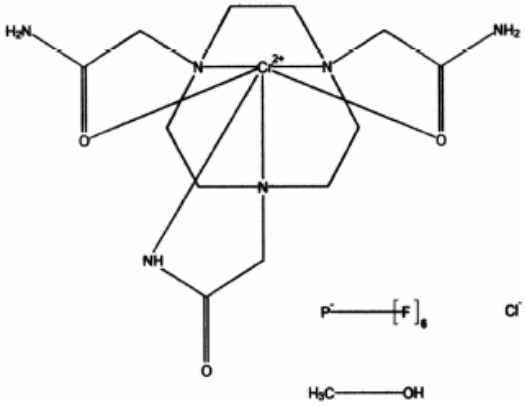
GIWRIN

Reference: T.Weyhermuller, K.Wieghardt, P.Chaudhuri (1996) *J.Chem.Soc.,Dalton Trans.*,3805

Formula: $C_{12}\,H_{23}\,Cr_1\,N_6\,O_3\,^{2+}$,$Cl_1\,^{1-}$,$F_6\,P_1\,^{1-}$,$C_1\,H_4\,O_1$

Compound Name: (1,4,7-tris(Carbamoylmethyl)-1,4,7-triazacyclononane)-chromium(III) chloride hexafluorophosphate methanol solvate

R-Factor (%): 4.39 **Temperature(K):** 100 **Density(g/cm^3):** 1.699



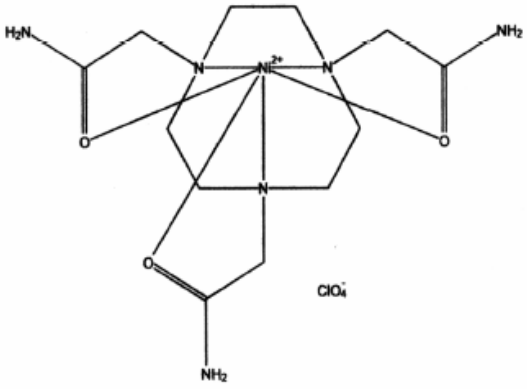
GIWRUZ

Reference: T.Weyhermuller, K.Wieghardt, P.Chaudhuri (1998) *J.Chem.Soc.,Dalton Trans.*,3805

Formula: $C_{12}\,H_{24}\,N_6\,Ni_1\,O_3\,^{2+}$,$2(Cl_1\,O_4\,^{1-})$

Compound Name: (1,4,7-tris(Carbamoylmethyl)-1,4,7-triazacyclononane)-nickel(II) diperchlorate

R-Factor (%): 6.62 **Temperature(K):** 295 **Density(g/cm^3):** 1.707



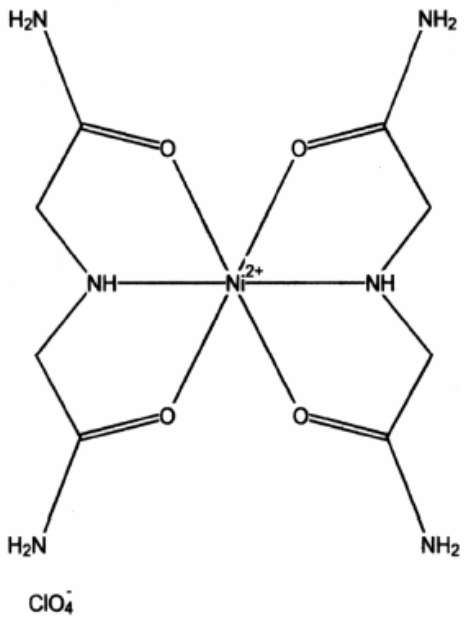
IDACNI

Reference: M.Sekizaki (1976) *Acta Crystallogr.,Sect.B:Struct.Crystallogr.Cryst.Chem.*,**32**,1568

Formula: $C_8\,H_{18}\,N_6\,Ni_1\,O_4\,^{2+}$,$2(Cl_1\,O_4\,^{1-})$

Compound Name: bis(Iminodiacetamide-N,O,O')-nickel(II) diperchlorate

R-Factor (%): 5.40 **Temperature(K):** 295 **Density(g/cm^3):** 1.843



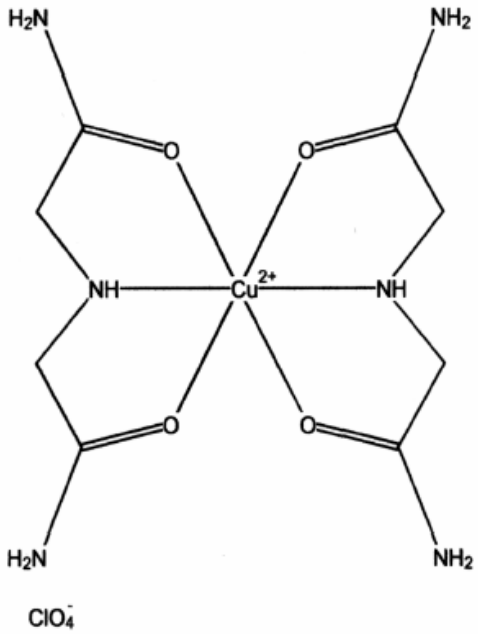
IMACUP

Reference: M.Sekizaki (1974) *Bull.Chem.Soc.Jpn.*,**47**,1447

Formula: $C_8\,H_{18}\,Cu_1\,N_6\,O_4\,^{2+}$,$2(Cl_1\,O_4\,^{1-})$

Compound Name: bis(Iminodiacetamide-N,O,O')-copper(II) diperchlorate

R-Factor (%): 8.00 **Temperature(K):** 295 **Density(g/cm^3):** 1.915



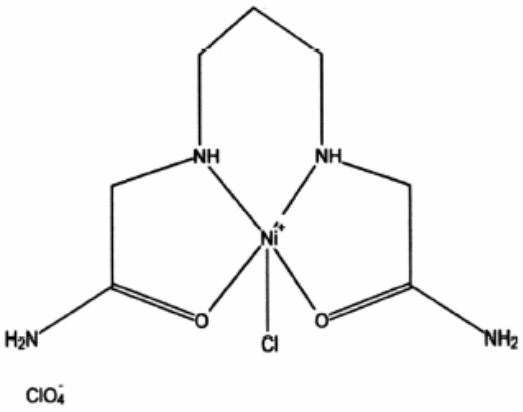
JAPJIT

Reference: Chung Chieh, Fang-Jy Wu, Sue-Lein Wang, Chin-Hohn Lan, Chung-Sun Chung (1989)
Acta Crystallogr.,Sect.C:Cryst.Struct.Commun.,**45**,1076

Formula: $C_7 H_{16} Cl_1 N_4 Ni_1 O_2^{1+}, Cl_1 O_4^{1-}$

Compound Name: Chloro-(3,7-diazanonanediamide)-nickel(II) perchlorate

R-Factor (%): 4.37 **Temperature(K):** 295 **Density(g/cm³):** 1.831



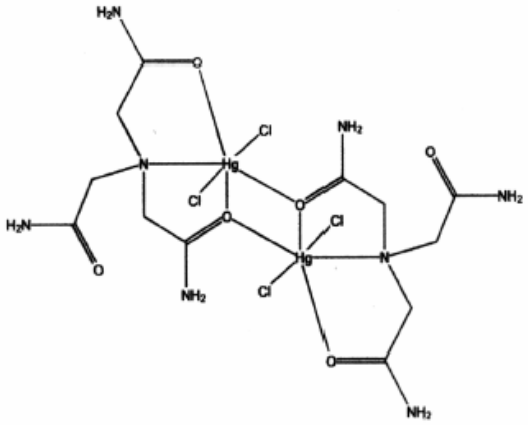
LEZDOJ

Reference: E.Skrzypczak-Jankun, D.A.Smith (1994)
Acta Crystallogr.,Sect.C:Cryst.Struct.Commun.,**50**,1585

Formula: $C_{12} H_{24} Cl_4 Hg_2 N_8 O_8$

Compound Name: bis(μ2-Nitrilo-N-triacetamide-N,O,O')-bis(dichloro-mercury(II))

R-Factor (%): 3.40 **Temperature(K):** 295 **Density(g/cm³):** 2.533



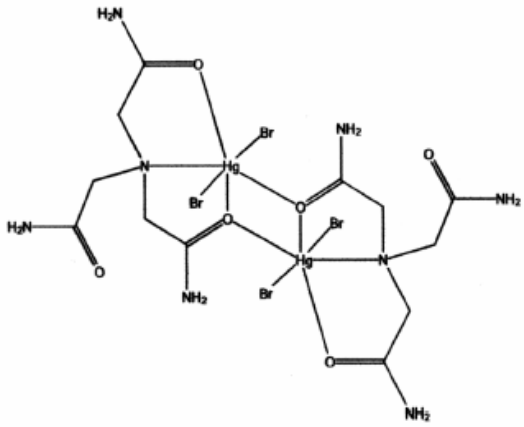
LEZDUP

Reference: E.Skrzypczak-Jankun, D.A.Smith (1994)
Acta Crystallogr.,Sect.C:Cryst.Struct.Commun.,**50**,1585

Formula: $C_{12} H_{24} Br_4 Hg_2 N_8 O_8$

Compound Name: bis(μ2-Nitrilo-N-triacetamide)-bis(dibromo-mercury(II))

R-Factor (%): 4.50 **Temperature(K):** 295 **Density(g/cm³):** 2.871



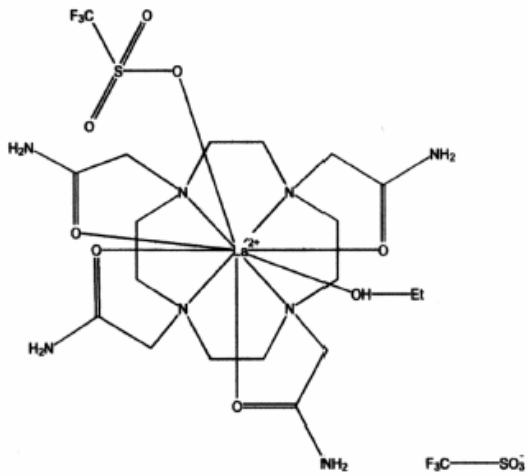
PIRSEO

Reference: S.Amin, J.R.Morrow, C.H.Lake, M.R.Churchill (1994)
Angew.Chem.,Int.Ed.Engl.,**33**,773

Formula: $C_{19} H_{36} F_3 La_1 N_8 O_8 S_1^{2+}, 2(C_1 F_3 O_3 S_1^{1-})$

Compound Name: Ethanolato-(1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetra-azacyclododecane)-(trifluoromethanesulfonato-O)-lanthanum bis(trifluoromethanesulfonate)

R-Factor (%): 2.60 **Temperature(K):** 295 **Density(g/cm³):** 1.778



QEZWEX

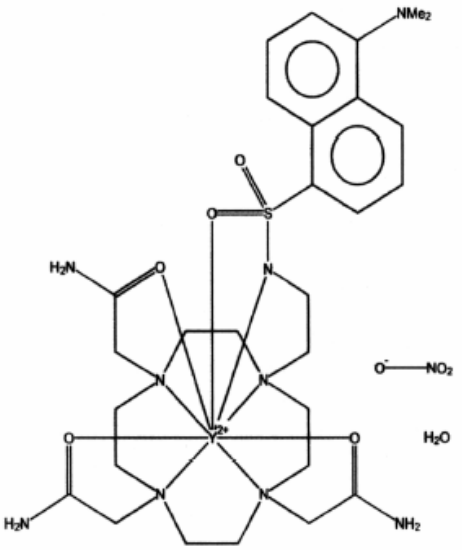
Reference: S.Aoki, H.Kawatani, T.Goto, E.Kimura, M.Shiro (2001) *J.Am.Chem.Soc.*,**123**,1123

Formula: $C_{28}H_{44}N_9O_5S_1Y_1{}^{2+},2(N_1O_3{}^{1-}),2.5(H_2O_1)$

Compound Name: (1-(2-(5-(Dimethylamino)naphthalenesulfonylamido)ethyl)-4,7,10-tris(carbamoylmethyl)-1,4,7,10-tetra-azacyclododecane)-yttrium(iii) dinitrate hydrate

Synonym: (1-(2-(5-(Dimethylamino)naphthalenesulfonylamido)ethyl)-4,7,10-tris(carbamoylmethyl)cyclen)-yttrium(iii) dinitrate hydrate

R-Factor (%): 9.93 **Temperature(K):** 103 **Density(g/cm^3):** 1.494



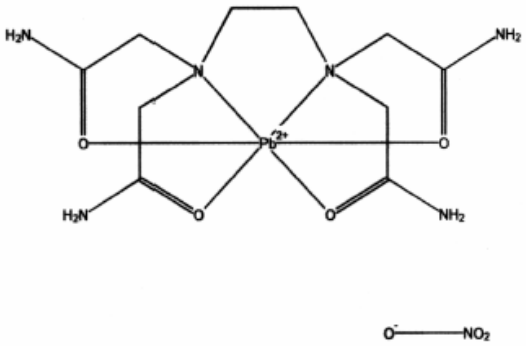
QIDKOD

Reference: E.S.Claudio, M.A.ter Horst, C.E.Forde, C.L.Stern, M.K.Zart, H.A.Godwin (2000) *Inorg.Chem.*,**39**,1391

Formula: $C_{10}H_{20}N_6O_4Pb_1{}^{2+},2(N_1O_3{}^{1-})$

Compound Name: (Ethylenediaminetetraacetamide)-lead(ii) dinitrate

R-Factor (%): 3.60 **Temperature(K):** 153 **Density(g/cm^3):** 2.318



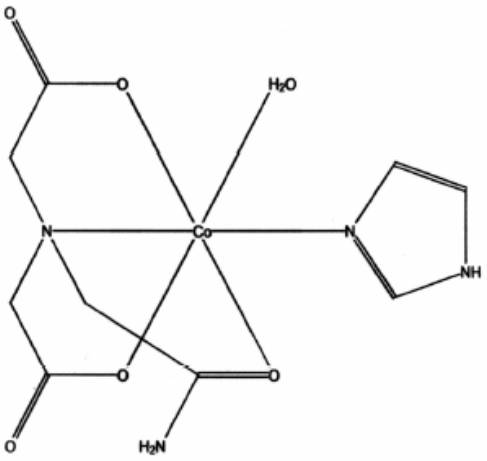
QIFQUR

Reference: E.Bugella-Altamirano, J.M.Gonzalez-Perez, A.G.Sicilia Zafra, J.Niclos-Gutierrez, A.Castineiras-Campos (2000) *Polyhedron*,**19**,2473

Formula: $C_9H_{14}Co_1N_4O_6.1.5(H_2O_1)$

Compound Name: Aqua-(N-(carbamoylmethyl)iminodiacetato)-(imidazole)-cobalt(ii) sesquihydrate

R-Factor (%): 6.30 **Temperature(K):** 295 **Density(g/cm^3):** 1.638



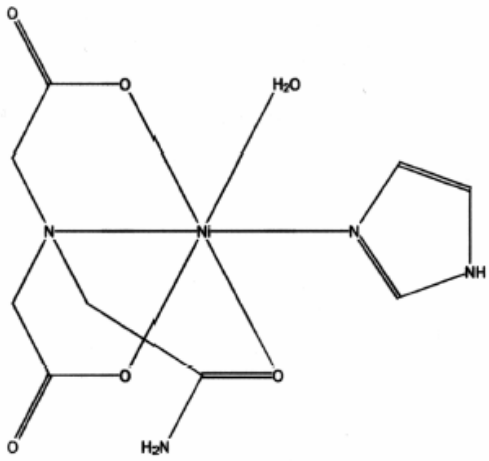
QIFRAY

Reference: E.Bugella-Altamirano, J.M.Gonzalez-Perez, A.G.Sicilia Zafra, J.Niclos-Gutierrez, A.Castineiras-Campos (2000) *Polyhedron*,**19**,2473

Formula: $C_9H_{14}N_4Ni_1O_6.1.5(H_2O_1)$

Compound Name: Aqua-(N-(carbamoylmethyl)iminodiacetato)-(imidazole)-nickel(ii) sesquihydrate

R-Factor (%): 5.41 **Temperature(K):** 295 **Density(g/cm^3):** 1.674



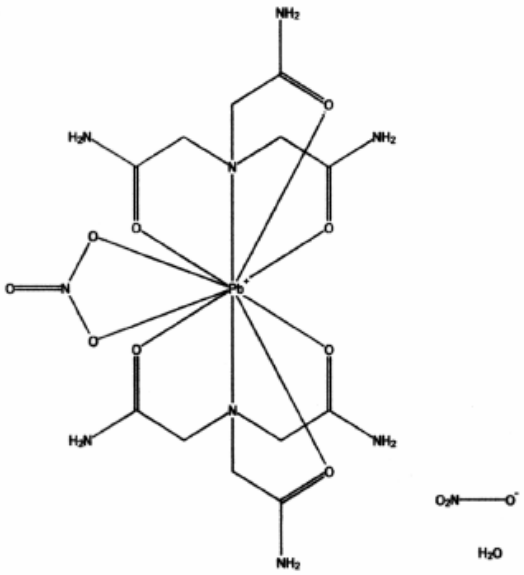
SOYZIP

Reference: D.A.Smith, S.Sucheck, A.A.Pinkerton (1992) *Chem.Commun.*,367

Formula: $C_{12}\ H_{24}\ N_8\ O_9\ Pb_1\ ^{1+},N_1\ O_3\ ^{1-},2(H_2\ O_1)$

Compound Name: bis(Nitrilotriacetamide-N,O,O',O'')-(nitrato-O,O')-lead nitrate dihydrate

R-Factor (%): 2.90 ***Temperature(K):*** 295 ***Density(g/cm³):*** 2.035



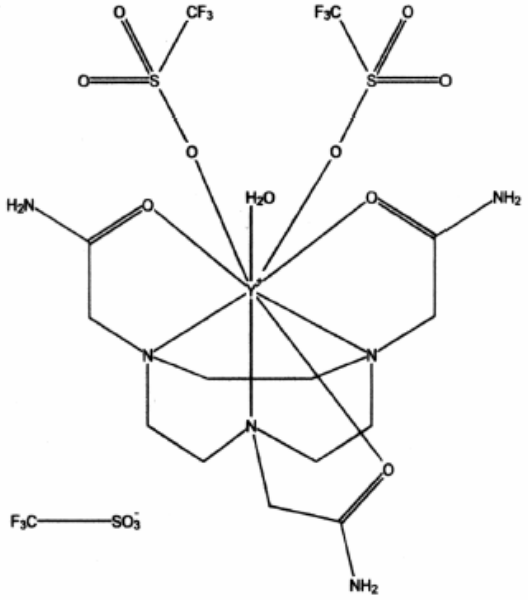
TOSREY

Reference: S.Amin, C.Marks, L.M.Toomey, M.R.Churchill, J.R.Morrow (1996) *Inorg.Chim.Acta*,246,99

Formula: $C_{14}\ H_{26}\ F_6\ N_6\ O_{10}\ S_2\ Y_1\ ^{1+},C_1\ F_3\ O_3\ S_1\ ^{1-}$

Compound Name: Aqua-bis(trifluoromethanesulfonato)-(1,4,7-tris(carbamoylmethyl)-1,4,7-triazacyclononane)-yttrium(iii) trifluoromethanesulfonate

R-Factor (%): 5.64 ***Temperature(K):*** 295 ***Density(g/cm³):*** 1.884



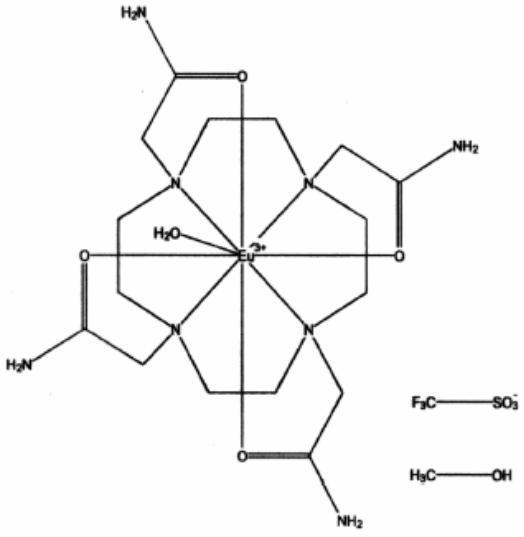
ZACXAC

Reference: S.Amin, D.A.Voss Junior, W.DeW.Horrocks Junior, C.H.Lake, M.R.Churchill, J.R.Morrow (1995) *Inorg.Chem.*,34,3294

Formula: $C_{16}\ H_{34}\ Eu_1\ N_8\ O_5\ ^{3+},3(C_1\ F_3\ O_3\ S_1\ ^{1-}),2(C_1\ H_4\ O_1)$

Compound Name: Aqua-(1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetra-azacyclododecane)-europium(iii) tris(trifluoromethanesulfonate) methanol solvate

R-Factor (%): 4.02 ***Temperature(K):*** 295 ***Density(g/cm³):*** 1.774



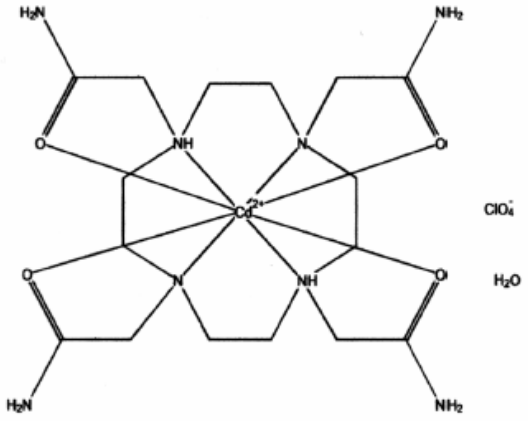
ZAGLIC

Reference: H.Maumela, R.D.Hancock, L.Carlton, J.H.Reibenspies, K.P.Wainwright (1995) *J.Am.Chem.Soc.*,117,6698

Formula: $C_{16}\ H_{34}\ Cd_1\ N_8\ O_4\ ^{2+},2(Cl_1\ O_4\ ^{1-}),1.5(H_2\ O_1)$

Compound Name: (1,4,7,10-tetrakis(Acetamido)-1,4,7,10-tetra-azacyclododecane-N,N',N'',N''',O,O',O'',O''')-cadmium(ii) diperchlorate sesquihydrate

R-Factor (%): 3.91 ***Temperature(K):*** 295 ***Density(g/cm³):*** 1.739



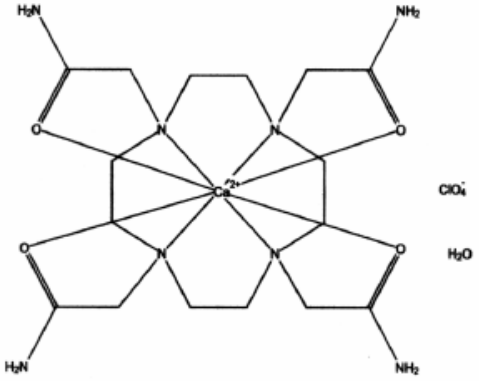
ZAGLOI

Reference: H.Maumela, R.D.Hancock, L.Carlton, J.H.Reibenspies, K.P.Wainwright (1995) *J.Am.Chem.Soc.*,117,6698

Formula: $C_{16}H_{32}Ca_1N_8O_4^{2+},2(Cl_1O_4^{1-}),2.5(H_2O_1)$

Compound Name: (1,4,7,10-tetrakis(Acetamido)-1,4,7,10-tetra-azacyclododecane-N,N',N'', N''',O,O',O'',O''')-calcium(II) diperchlorate hydrate

R-Factor (%): 10.94 **Temperature(K):** 295 **Density(g/cm^3):** 1.595



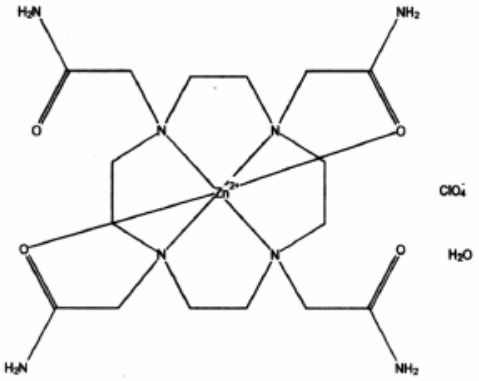
ZAGLUO

Reference: H.Maumela, R.D.Hancock, L.Carlton, J.H.Reibenspies, K.P.Wainwright (1995) *J.Am.Chem.Soc.*,117,6698

Formula: $C_{16}H_{32}N_8O_4Zn_1^{2+},2(Cl_1O_4^{1-}),H_2O_1$

Compound Name: (1,4,7,10-tetrakis(Acetamido)-1,4,7,10-tetra-azacyclododecane-N,N',N'', N''',O,O'')-zinc(II) diperchlorate monohydrate

R-Factor (%): 7.67 **Temperature(K):** 295 **Density(g/cm^3):** 1.700



ZULGAO

Reference: M.Sivak, J.Tyrselova, F.Pavelcik, J.Marek (1996) *Polyhedron*,15,1057

Formula: $C_6H_8N_2O_8V_1^{1-},K_1^{1+},4(H_2O_1)$

Compound Name: Potassium (N-(carbamoylmethyl)iminodiacetato)-oxo-peroxo-vanadium(v) tetrahydrate

R-Factor (%): 3.48 **Temperature(K):** 121 **Density(g/cm^3):** 1.808